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(54) Title: REPORTER GENE SYSTEM FOR USE IN CELL-BASED ASSESSMENT OF INHIBITORS OF THE HEPATITIS C VIRUS PROTEASE

(57) Abstract

A cell-based assay system in which the detection of the reporter gene activity, or secreted alkaline phosphatase (SEAP), is dependent upon the protease activity of the Hepatitis C virus NS3 gene product. This system can be used to assess the activity of candidate protease inhibitors in a mammalian cell-based assay system. The assay system is simpler than previously described assays due to the use of SEAP which allows the reporter gene activity to be quantified by measuring the amount of secreted gene product in the cell media by monitoring the conversion of luminescent or colorimetric alkaline phosphatase substrate.

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Reporter Gene System For Use In Cell-Based Assessment Of Inhibitors Of The Hepatitis C Virus Protease

Technical and Industrial Applicability of Invention

A cell-based assay system in which the detection of reporter gene activity (secreted alkaline phosphatase or SEAP) is dependent upon active Hepatitis C virus (HCV) NS3 protease. The assay system is useful in the *in vitro* screening, in a mammalian cell-based assay, of potential protease inhibiting molecules useful in the treatment of HCV. The advantages of using SEAP over more routinely used reporter genes such as beta-galactosidase or luciferase, is that a cell lysis step is not required since the SEAP protein is secreted out of the cell. The absence of a cell lysis step decreases intra- and inter-assay variability as well as makes the assay easier to perform then earlier assays.

Background of The Invention

HCV is one of the major causes of parenterally transmitted non-A, non-B hepatitis worldwide. HCV is now known as the etiologic agent for Non-A Non-B hepatitis throughout the world. Mishiro et al., U.S. Patent No. 5,077,193; Mishiro et al., U.S. Patent No. 5,176,994; Takahashi et al, U.S. Patent No. 5,032,511; Houghton et al., U.S. Patent Nos. 5,714,596 and 5,712,088; as well as (M. Houghton, *Hepatitis C Viruses*, p.1035-1058 in B.N. Fields et al.(eds.), Field's Virology (3d. ed. 1996). HCV infection is characterized by the high rate (>70%) with which acute infection progresses to chronic infection (Alter, M. J. 1995. Epidemiology of hepatitis C in the west. Sem. Liver Dis. 15:5-14.). Chronic HCV infection may lead to progressive liver injury, cirrhosis, and in some cases, hepatocellular carcinoma. Currently, there are no specific antiviral agents available for the treatment of HCV infection. Although alpha interferon therapy is often used in the treatment of HCV-induced moderate or severe liver disease, only a minority of patients exhibit a sustained response Saracco, G. et al., J. Gastroenterol. Hepatol. 10:668-673 1995. Additionally, a vaccine to prevent HCV infection is not yet available and it remains uncertain whether vaccine development will be complicated by the existence of multiple HCV genotypes as well as viral

variation within infected individuals Martell, M. et al., J. Virol. 66:3225-3229 1992; Weiner, et al., Proc. Natl. Acad. Sci. 89:3468-3472 1992. The presence of viral heterogeneity may increase the likelihood that drug resistant virus will emerge in infected individuals unless antiviral therapy effectively suppresses virus replication. Most recently, several of the HCV encoded enzymes, specifically the NS3 protease and NS5B RNA polymerase, have been the focus of intensive research, in vitro screening, and/or rational drug design efforts.

HCV has been classified in the flavivirus family in a genus separate from that of the flaviviruses and the pestiviruses. Rice, C. M., in B. N. Fields and P. M. Knipe (eds.), Virology, 3rd edn., p. 931-959;1996 Lippincott-Raven, Philadelphia, PA. Although the study of HCV replication is limited by the lack of an efficient cell-based replication system, an understanding of replicative events has been inferred from analogies made to the flaviviruses, pestiviruses, and other positive strand RNA viruses. The HCV virus has a 9.4 kb single positive-strand RNA genome encoding over 3,000 amino acids. The genome expresses over 10 structural and non-structural proteins. Post-translational processing of the viral genome requires cleavage by two proteases. As in the pestiviruses, translation of the large open reading frame occurs by a cap-independent mechanism and results in the production of a polyprotein of 3010-3030 amino acids. Proteolytic processing of the structural proteins (the nucleocapsid protein or core (C)) and two envelope glycoproteins, E1 and E2 is accomplished by the action of host cell signal peptidases. Santolini, E., et al., J. Virol. 68:3631-3641, 1994; Ralston, R., et al., J. Virol. 67:6753-6761 1993. Cleavage of the nonstructural proteins (NS4A, NS4B, NS5A, and NS5B) is mediated by the action of the NS2/3 protease or the NS3 protease. Grakoui, A. et al., J. Virol. 67:2832-2843 1993; Hirowatari, Y., et al., Anal. Biochem. 225:113-120 1995; Bartenschlager, R. et al., J. Virol. 68:5045-5055 1994; Eckart, M. R., et al., Biochem. Biophys. Res. Comm. 192:399-406 1993; Grakoui, A., et al., J. Virol. 67:2832-2843 1993; Tomei, L., et al., J. Virol. 67:4017-40261993; NS4A is a cofactor for NS3 and NS5B is an RNA dependent RNA polymerase. Bartenschlager, R. et al., (1994); Failla, C.,et al., . J. Virol. 68:3753-3760 1994; Lin, C. et al., Proc. Natl. Acad. Sci. 92:7622-

7626 1995; Behrens, S.-E., et al., EMBO J. 15:12-22 1996. Functions for the NS4B and NS5A proteins have yet to be defined.

The NS2/3 is a metalloprotease and has been shown to mediate cleavage at the 2/3 junction site Grakoui, et al. (1993); Hijikata, M., et al., J. Virol. 67:4665-4675 1993. In contrast, the NS3 protease is required for multiple cleavages within the nonstructural segment of the polyprotein, specifically the 3/4A, 4A/4B, 4B/5A, and 5A/5B junction sites Bartenschlager et al. (1993); Eckart, M. R., et al., Biochem. Biophys. Res. Comm. 192:399-406 1993; Grakoui et al. (1993); Tomei et al. (1994). More recently, it is thought that the NS2/3 protease might actually be part of the HCV NS3 protease complex even though they have two functionally distinct activities. Although NS3 protease is presumed to be essential for HCV viability, definitive proof of its necessity has been hampered by the lack of an infectious molecular clone that can be used in cell-based experiments. However, recently two independent HCV infectious molecular clones have been developed and have been shown to replicate in chimpanzees. Kolykhalov, A. A., et al., Science 277:570-574 1997; Yanagi, M., et al., Proc. Natl. Acad. Sci. 94:8738-8743 1997. The requirement for NS3 in the HCV life cycle may be validated in these clones by using oligo nucleotide-mediated site directed mutagenesis to inactivate the NS3 catalytic serine residue and then determining whether infectious virus is produced in chimpanzees. Until these experiments are performed, the necessity of NS3 is inferred from cell-based experiments using the related yellow fever (YFV) and bovine viral diarrhea (BVDV) viruses. Mutagenesis of the YFV and BVDV NS3 protease homologs has shown that NS3 serine protease activity is essential for YFV and BVDV replication. Chambers, T. J., et al., Proc. Natl. Acad. Sci. 87:8898-8902 1990; Xu, J., et al., J. Virol. 71:5312-5322 1997.

In general, when investigators screen potential anti-viral compounds for inhibitory activity, it usually involves initial *in vitro* testing of putative enzyme inhibitors followed by testing the compounds on actual infected cell lines and animals. It is obvious that working with live virus in large scale screening activities can be inherently dangerous and problematic. While final testing of putative inhibitors in infected cells and animals is still necessary for preclinical drug development, for initial screening of candidate molecules, such work is cost-prohibitive and unnecessary. Furthermore, the inability to grow HCV in tissue culture in a reproducible quantitative

manner prevents the evaluation of potential antiviral agents for HCV in a standard antiviral cytopathic effect assay. In response to this real need in the industry, development of non-infectious, cell-based, screening systems is essential.

For example, Hirowatari, et al. developed a reporter assay system, *inter alia*, that involves the transfection of mammalian cells with two eukaryotic expression plasmids. Hirowatari, et al., Anal. Biochem. 225:113-120 1995. One plasmid has been constructed to express a polyprotein that encompasses the HCV NS2-NS3 domains fused in frame to an NS3 cleavage site followed by the HTLV-1 TAX1 protein. A second plasmid has been constructed to have the expression of the chloramphenicol acetyltransferase (CAT) reporter gene under the control of the HTLV-1 LTR. Thus when COS cells are transfected with both plasmids, NS3-mediated cleavage of the TAX1 protein from the NS2-NS3-TAX1 polyprotein allows the translocation of TAX1 to the nucleus and subsequent activation of CAT transcription from the HTLV-1 LTR. CAT activity can be measured by assaying the acetylation of ^{14}C-chloramphenicol through chromatographic or immunological methods. In the CAT assay generally, cell extracts are incubated in a reaction mix containing ^{14}C- or ^{3}H-labeled chloramphenicol and n-Butyryl Coenzyme A. The CAT enzyme transfers the n-butyryl moiety of the cofactor to chloramphenicol. For a radiometric scintillation detection (LSC) assay, the reaction products are extracted with a small volume of xylene. The n-butyryl chloramphenicol partitions mainly into the xylene phase, while unmodified chloramphenicol remains predominantly in the aqueous phase. The xylene phase is mixed with a liquid scintillant and counted in a scintillation counter. The assay can be completed in as little as 2-3 hours, is linear for nearly three orders of magnitude, and can detect as little as 3×10^{-4} units of CAT activity. CAT activity also can be analyzed using thin layer chromatography (TLC). This method is more time-consuming than the LSC assay, but allows visual confirmation of the data.

Similarly, the other patents of Houghton, et al., U.S. Patent No. 5,371,017, U.S. Patent No. 5,585,258, U.S. Patent No. 5,679,342 and U.S. Patent No. 5,597,691 or Jang et al. WO 98/00548 all disclose a cloned NS3 protease or portion fused to a second gene encoding for a protein which a surrogate expression product can be detected for example, in the '017 patent of Houghton, b-galactosidase, superoxide dismutase, ubiquitin or in Jang, the expression is measured by the proliferation of

poliovirus in cell culture) and its use for candidate screening. It is unclear in the Houghton, et al. patents, however, whether the protease described in the specification is the NS2/3 metalloprotease or NS3 serine protease. Although the serine protease is claimed, the experimental data show putative cleavage of the N-terminal SOD fusion partner at the NS2/3 junction, a function which recently has been deemed to be the domain of the NS2/3 metalloprotease (Rice, C.M., et al., Proc. Nat. Acad. Sci. 90:10583-10587 (1993)). Furthermore, an active soluble NS3 serine protease is not disclosed in the Houghton, et al. patents, but a insoluble protein derived from *E. coli* inclusion bodies and which was N-terminally sequenced. For purposes of the present invention the term "NS2 protease" will refer to the enzymatic activity associated with the NS2/3 metalloprotease as defined by Rice et al., and the term "NS3 protease" will refer to the serine protease located within the NS3 region of the HCV genome.

De Francesco et al., U.S. Patent No. 5,739,002, also describes a cell free in vitro system for testing candidates which activate or inhibit NS3 protease by measuring the amount of cleaved substrate. Hirowatari et al. (1995) discloses another HCV NS3 protease assay, however, it differs from the present invention in several aspects, including the reporter gene, the expression plasmid constructs, and the method of detection. Recently, Cho et al. describe a similar SEAP reporter system for assaying HCV NS3 protease which also differs in its structure and function from the present invention. Cho et al., J. Virol. Meth. 72:109-115 1998. Also of interest is a NS3 protease assay system developed by Chen et al. in WO 98/37180. In the Chen et al. application, a fusion protein is described which uses NS3 protease polypeptide or various truncation analogs fused to the NS4A polypeptide or various truncation analogs and is not autocleavable. The fusion protein is then incubated with known substrates with or without inhibitors to screen for inhibitory effect.

There are a number of problems inherent in all the abovementioned assay systems. For example, the reporter gene product or analyte is many steps removed from the initial NS3 protease cleavage step, the cells used in the assay system are prokaryotic or Yeast based and must be lysed before the reporter gene product can be measured, and the surrogate marker is proliferation of live virus. All of these problems are overcome in the present invention as summarized below.

Summary of Invention

The present invention describes a reporter gene system for use in the cell based assessment of inhibitors of the HCV protease. Applicants point out that throughout the description of this invention, the reference to specific non-structural (NS) regions or domains of the HCV genome are functional definitions and correspond approximately to the defined sequence locations described by C.M. Rice and others. The present invention discloses the co-transfection of a target cell line with a viral vector which has been engineered to express from the T7 RNA polymerase promoter and a recombinant plasmid or viral vector which has been engineered to express a polyprotein that includes NS3 HCV serine protease and the secreted human placental alkaline phosphatase (SEAP) gene (Berger et al. 1988) under control of the T7 promoter. The present invention was designed to have a linkage between the detection of reporter gene activity and NS3 serine protease activity through construction of a segment of the HCV gene encoding the NS2-NS3-NS4A-NS4B'-sequence linked to the SEAP reporter.

Detection of NS3 protease activity is accomplished by having the release and hence, the subsequent detection, of the SEAP reporter gene to be dependent upon NS3 serine protease activity. In a preferred embodiment, the target cell line is first infected with a viral vector that expresses the T7 RNA polymerase followed by either co-infection with a second viral vector that encodes the NS3 HCV protease/SEAP polyprotein, or transfection with a plasmid that contains the same NS3/SEAP gene elements.

The SEAP enzyme is a truncated form of human placental alkaline phosphatase, in which the cleavage of the transmembrane domain of the protein allows it to be secreted from the cells into the surrounding media. SEAP activity can be detected by a variety of methods including, but not limited to, measurement of catalysis of a fluorescent substrate, immunoprecipitation, HPLC, and radiometric detection. The luminescent method is preferred due to its increased sensitivity over colorimetric detection methods, and such an assay kit is available from Tropix®. The advantages of using SEAP over more routinely used reporter genes such as beta-galactosidase or luciferase, is that a cell lysis step is not required since the SEAP protein is secreted out of the cell. The absence of a cell lysis step decreases intra-

and inter-assay variability as well as makes the assay easier to perform then earlier assays in the prior art. When both the T7 promoter and NS3/SEAP constructs are present, SEAP can be detected in the cell medium within the usual viral assay timeframe of 24-48 hours, however, the timeframe should not be read as a limitation because it is theoretically possible to detect the SEAP in the media only a few hours after transfection. The medium can then be collected and analyzed . Various examples illustrating the use of this composition and method will be detailed below.

Brief Description of the Drawings

Figure 1 illustrates schematically the Vaccinia Virus NS3/SEAP System gene construct.

Figure 1B illustrates schematically the Plasmid/Vaccinia Virus NS3/SEAP assay.

Figure 2 illustrates schematically how the assay operates.

Figure 3 illustrates schematically the DI/DR Assay.

Figure 4A and 4B shows the SEAP activity dose response curve for a representative plasmid/virus assay.

Figure 5 shows an experimental 96 well plate diagram for the SEAP protocol on Day 1 in Example 3.

Figure 6 shows an experimental 96 well plate diagram for the SEAP protocol on Day 2 in Example 3.

Figure 7 shows SEAP activity and Cytotoxicity data for Example 4.

Figure 8 shows a summary of DI/DR assay data.

Figure 9 illustrates the experimental plate set-up for Example 2.

Detailed Description of a Preferred Embodiment of the Invention

The practice of this invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA manipulation and production, virology and immunology, which are within the skill of the art. Such techniques are explained fully in the literature: Sambrook, *Molecular Cloning; A Laboratory Manual,* Second Edition (1989); *DNA Cloning,* Volumes I and II (D. N. Glover, Ed. 1985); *Oligonucleotide Synthesis* (M. J. Gait, Ed. 1984); *Nucleic Acid Hybridization* (B. D. Hames and S. I. Higgins, Eds. 1984); *Transcription and*

Translation (B. D. Hames and S. I. Higgins, Eds. 1984); *Animal Cell Culture* (R. I. Freshney, Ed. 1986); *Immobilized Cells and Enzymes* (IRL Press, 1986); B. Perbal, *A Practical Guide to Molecular Cloning* (1984); *Gene Transfer Vectors for Mammalian Cells* (J. H. Miller and M. P. Calos, Eds. 1987, Cold Spring Harbor Laboratory); *Methods in Enzymology,* Volumes 154 and 155 (Wu and Grossman, and Wu, Eds., respectively), (Mayer and Walker, Eds.) (1987); *Immunochemical Methods in Cell and Molecular Biology* (Academic Press, London), Scopes, (1987), *Expression of Proteins in Mammalian Cells Using Vaccinia Viral Vectors* in *Current Protocols in Molecular Biology*, Volume 2 (Frederick M. Ausubel, et al., Eds.)(1991). All patents, patent applications and publications mentioned herein, both supra and infra, are hereby incorporated by reference.

Both prokaryotic and eukaryotic host cells are useful for expressing desired coding sequences when appropriate control sequences compatible with the designated host are used. Among prokaryotic hosts, *E. coli* is most frequently used. Expression control sequences for prokaryotes include promoters, optionally containing operator portions, and ribosome binding sites. Transfer vectors compatible with prokaryotic hosts are commonly derived from, for example, pBR322, a plasmid containing operons conferring ampicillin and tetracycline resistance, and the various pUC vectors, which also contain sequences conferring antibiotic resistance markers. These plasmids are commercially available. The markers may be used to obtain successful transformants by selection. Commonly used prokaryotic control sequences include the β-lactamase (penicillinase) and lactose promoter systems (Chang et al, *Nature* (1977) 198:1056), the tryptophan (trp) promoter system (Goeddel et al, *Nuc Acids Res* (1980) 8:4057) and the lambda-derived P_L promoter and N gene ribosome binding site (Shimatake et al, *Nature* (1981) 292:128) and the hybrid tac promoter (De Boer et al, *Proc Nat Acad Sci USA* (1983) 292:128) derived from sequences of the trp and lac UV5 promoters. The foregoing systems are particularly compatible with *E. coli;* if desired, other prokaryotic hosts such as strains of Bacillus or Pseudomonas may be used, with corresponding control sequences.

Eukaryotic hosts include without limitation yeast and mammalian cells in culture systems. Yeast expression hosts include Saccharomyces, Klebsiella, Picia, and the like. *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis* and *K. lactis* are the most commonly used yeast hosts, and are convenient fungal hosts.

Yeast-compatible vectors carry markers which permit selection of successful transformants by conferring prototrophy to auxotrophic mutants or resistance to heavy metals on wild-type strains. Yeast compatible vectors may employ the 2 μ origin of replication (Broach et al, *Meth Enzymol (*1983) 101:307), the combination of CEN3 and ARS1 or other means for assuring replication, such as sequences which will result in incorporation of an appropriate fragment into the host cell genome. Control sequences for yeast vectors are known in the art and include promoters for the synthesis of glycolytic enzymes (Hess et al, *J Adv Enzyme Reg* (1968) 7:149; Holland et al, *Biochem* (1978), 17:4900), including the promoter for 3-phosphoglycerate kinase (R. Hitzeman et al, *J Biol Chem* (1980) 255:2073). Terminators may also be included, such as those derived from the enolase gene (Holland, *J Biol Chem* (1981) 256:1385).

Mammalian cell lines available as hosts for expression are known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including HeLa cells, Chinese hamster ovary (CHO) cells, baby hamster kidney (BHK) cells, BSC 1 cells, CV1 cells, and a number of other cell lines. Suitable promoters for mammalian cells are also known in the art and include vital promoters such as that from Simian Virus 40 (SV40) (Fiers et al, *Nature* (1978) 273:113), Rous sarcoma virus (RSV), adenovirus (ADV), and bovine papilloma virus (BPV). Mammalian cells may also require terminator sequences and poly-A addition sequences. Enhancer sequences which increase expression may also be included, and sequences which promote amplification of the gene may also be desirable (for example methotrexate resistance genes). These sequences are known in the art.

Vectors suitable for replication in mammalian cells are known in the art, and may include vital replicons, or sequences which insure integration of the appropriate sequences encoding HCV epitopes into the host genome. For example, another vector used to express foreign DNA is Vaccinia virus. In this case the heterologous DNA is inserted into the Vaccinia genome and transcription can be directed by either endogenous vaccinia promoters or exogenous non-vaccinia promoters (e.g. T7 retroviral promoter) known to those skilled in the art, depending on the characteristics of the constructed vector. Techniques for the insertion of foreign DNA into the vaccinia virus genome are known in the art, and may utilize, for example, homologous recombination. The heterologous DNA is generally inserted into a gene which is non-

essential to the virus, for example, the thymidine kinase gene (tk), which also provides a selectable marker. Plasmid vectors that greatly facilitate the construction of recombinant viruses have been described (see, for example, Mackett et al, *J Virol* (1984) 49:857; Chakrabarti et al, *Mol Cell Biol* (1985) 5:3403; Moss, in GENE TRANSFER VECTORS FOR MAMMALIAN CELLS (Miller and Calos, eds., Cold Spring Harbor Laboratory, N.Y., 1987), p. 10). Expression of the HCV polypeptide then occurs in cells or animals which are infected with the live recombinant vaccinia virus.

In order to detect whether or not the HCV polypeptide is expressed from the vaccinia vector, BSC 1 cells may be infected with the recombinant vector and grown on microscope slides under conditions which allow expression. The cells may then be acetone-fixed, and immunofluorescence assays performed using serum which is known to contain anti-HCV antibodies to a polypeptide(s) encoded in the region of the HCV genome from which the HCV segment in the recombinant expression vector was derived.

Other systems for expression of eukaryotic or vital genomes include insect cells and vectors suitable for use in these cells. These systems are known in the art, and include, for example, insect expression transfer vectors derived from the baculovirus *Autographa californica* nuclear polyhedrosis virus (AcNPV), which is a helper-independent, viral expression vector. Expression vectors derived from this system usually use the strong viral polyhedron gene promoter to drive expression of heterologous genes. Currently the most commonly used transfer vector for introducing foreign genes into AcNPV is pAc373 (see PCT WO89/046699 and U.S. Ser. No. 7/456,637). Many other vectors known to those of skill in the an have also been designed for improved expression. These include, for example, pVL985 (which alters the polyhedron start codon from ATG to ATT, and introduces a BamHI cloning site 32 bp downstream from the ATT; See Luckow and Summers, *Virol* (1989) 17:31). AcNPV transfer vectors for high level expression of non-fused foreign proteins are described in co-pending applications PCT WO89/046699 and U.S. Ser. No. 7/456,637. A unique BamHI site is located following position -8 with respect to the translation initiation codon ATG of the polyhedron gene. There are no cleavage sites for SmaI, PstI, BglII, XbaI or SstI. Good expression of non-fused foreign proteins usually requires foreign genes that ideally have a short leader sequence containing suitable translation

initiation signals preceding an ATG start signal. The plasmid also contains the polyhedron polyadenylation signal and the ampicillin-resistance (amp) gene and origin of replication for selection and propagation in *E. coli.*

Methods for the introduction of heterologous DNA into the desired site in the baculovirus virus are known in the art. (See Summer and Smith, Texas Agricultural Experiment Station Bulletin No. 1555; Smith et al, *Mol. Cell Biol.* (1983) 3:2156–2165; and Luckow and Summers, *Virol.* (1989) 17:31). For example, the heterologous DNA can be inserted into a gene such as the polyhedron gene by homologous recombination, or into a restriction enzyme site engineered into the desired baculovirus gene. The inserted sequences may be those which encode all or varying segments of the polyprotein, or other orfs which encode viral polypeptides. For example, the insert could encode the following numbers of amino acid segments from the polyprotein: amino acids 1–1078; amino acids 332–662; amino acids 406–662; amino acids 156–328, and amino acids 199–328.

The signals for post-translational modifications, such as signal peptide cleavage, proteolytic cleavage, and phosphorylation, appear to be recognized by insect cells. The signals required for secretion and nuclear accumulation also appear to be conserved between the invertebrate cells and vertebrate cells. Examples of the signal sequences from vertebrate cells which are effective in invertebrate cells are known in the art, for example, the human interleukin-2 signal ($IL2_S$) which signals for secretion from the cell, is recognized and properly removed in insect cells.

Transformation may be by any known method for introducing polynucleotides into a host cell, including, for example packaging the polynucleotide in a virus and transducing a host cell with the virus, and by direct uptake of the polynucleotide. The transformation procedure used depends upon the host to be transformed. Bacterial transformation by direct uptake generally employs treatment with calcium or rubidium chloride (Cohen, *Proc. Nat. Acad. Sci. USA* (1972) 69:2110; T. Maniatis et at, “Molecular Cloning; A Laboratory Manual” (Cold Spring Harbor Press, Cold Spring Harbor, N.Y., 1982). Yeast transformation by direct uptake may be carried out using the method of Hinnen et al, *Proc. Nat. Acad. Sci. USA* (1978) 75:1929. Mammalian transformations by direct uptake may be conducted using the calcium phosphate precipitation method of Graham and Van der Eb, *Virol.* (1978) 52:546, or the various

known modifications thereof. Other methods for introducing recombinant polynucleotides into cells, particularly into mammalian cells, include dextran-mediated transfection, calcium phosphate mediated transfection, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the polynucleotides into nuclei.

Vector construction employs techniques which are known in the art. Site-specific DNA cleavage is performed by treating with suitable restriction enzymes under conditions which generally are specified by the manufacturer of these commercially available enzymes. In general, about 1 mg of plasmid or DNA sequence is cleaved by 1 unit of enzyme in about 20 mL buffer solution by incubation for 1–2 hr at 37° C. After incubation with the restriction enzyme, protein is removed by phenol/chloroform extraction and the DNA recovered by precipitation with ethanol. The cleaved fragments may be separated using polyacrylamide or agarose gel electrophoresis techniques, according to the general procedures described in *Meth. Enzymol.* (1980) 65:499–560.

Sticky-ended cleavage fragments may be blunt ended using *E. coli* DNA polymerase I (Klenow fragment) with the appropriate deoxynucleotide triphosphates (dNTPs) present in the mixture. Treatment with S1 nuclease may also be used, resulting in the hydrolysis of any single stranded DNA portions.

Ligations are carried out under standard buffer and temperature conditions using T4 DNA ligase and ATP; sticky end ligations require less ATP and less ligase than blunt end ligations. When vector fragments are used as part of a ligation mixture, the vector fragment is often treated with bacterial alkaline phosphatase (BAP) or calf intestinal alkaline phosphatase to remove the 5′-phosphate, thus preventing re-ligation of the vector. Alternatively, restriction enzyme digestion of unwanted fragments can be used to prevent ligation. Ligation mixtures are transformed into suitable cloning hosts, such as *E. coli,* and successful transformants selected using the markers incorporated (e.g., antibiotic resistance), and screened for the correct construction.

Synthetic oligonucleotides may be prepared using an automated oligonucleotide synthesizer as described by Warner, *DNA* (1984) 3:401. If desired, the

synthetic strands may be labeled with ^{32}P by treatment with polynucleotide kinase in the presence of ^{32}P-ATP under standard reaction conditions.

DNA sequences, including those isolated from cDNA libraries, may be modified by known techniques, for example by site directed mutagenesis (see e.g., Zoller, *Nuc. Acids Res.* (1982) 10:6487). Briefly, the DNA to be modified is packaged into phage as a single stranded sequence, and converted to a double stranded DNA with DNA polymerase, using as a primer a synthetic oligonucleotide complementary to the portion of the DNA to be modified, where the desired modification is included in the primer sequence. The resulting double stranded DNA is transformed into a phage-supporting host bacterium. Cultures of the transformed bacteria which contain copies of each strand of the phage are plated in agar to obtain plaques. Theoretically, 50% of the new plaques contain phage having the mutated sequence, and the remaining 50% have the original sequence. Replicates of the plaques are hybridized to labeled synthetic probe at temperatures and conditions which permit hybridization with the correct strand, but not with the unmodified sequence. The sequences which have been identified by hybridization are recovered and cloned.

DNA libraries may be probed using the procedure of Grunstein and Hogness *Proc. Nat. Acad. Sci. USA* (1975) 73:3961. Briefly, in this procedure the DNA to be probed is immobilized on nitrocellulose filters, denatured, and pre-hybridized with a buffer containing 0–50% formamide, 0.75M NaCl, 75 mM Na citrate, 0.02% (wt/v) each of bovine serum albumin, polyvinylpyrrolidone, and Ficoll®, 50 mM NaH_2PO_4 (pH 6.5), 0.1% SDS, and 100 m g/mL carrier denatured DNA. The percentage of formamide in the buffer, as well as the time and temperature conditions of the pre-hybridization and subsequent hybridization steps depend on the stringency required. Oligomeric probes which require lower stringency conditions are generally used with low percentages of formamide, lower temperatures, and longer hybridization times. Probes containing more than 30 or 40 nucleotides, such as those derived from cDNA or genomic sequences generally employ higher temperatures, e.g., about 40°–42° C., and a high percentage formamide, e.g., 50%. Following pre-hybridization, 5′-^{32}P-labeled oligonucleotide probe is added to the buffer, and the filters are incubated in this mixture under hybridization conditions. After washing, the treated filters are subjected to autoradiography to show the location of the hybridized probe; DNA in

corresponding locations on the original agar plates is used as the source of the desired DNA.

For routine vector constructions, ligation mixtures are transformed into *E. coli* strain HB101 or other suitable hosts, and successful transformants selected by antibiotic resistance or other markers. Plasmids from the transformants are then prepared according to the method of Clewell et al, *Proc. Nat. Acad. Sci. USA* (1969) 62:1159, usually following chloramphenicol amplification (Clewell, *J. Bacteriol.* (1972) 110:667). The DNA is isolated and analyzed, usually by restriction enzyme analysis and/or sequencing. Sequencing may be performed by the dideoxy method of Sanger et at, *Proc. Nat. Acad. Sci. USA* (1977) 74:5463, as further described by Messing et at, *Nuc. Acids Res.* (1981) 9:309, or by the method of Maxam et at, *Meth. Enzymol.* (1980) 65:499. Problems with band compression, which are sometimes observed in GC-rich regions, were overcome by use of T-deazoguanosine according to Barr et al, *Biotechniques* (1986) 4:428.

Target plasmid sequences are replicated by a polymerizing means which utilizes a primer oligonucleotide to initiate the synthesis of the replicate chain. The primers are selected so that they are complementary to sequences of the plasmid. Oligomeric primers which are complementary to regions of the sense and antisense strands of the plasmids can be designed from the plasmid sequences already known in the literature.

The primers are selected so that their relative positions along a duplex sequence are such that an extension product synthesized from one primer, when it is separated from its template (complement), serves as a template for the extension of the other primer to yield a replicate chain of defined length.

The primer is preferably single stranded for maximum efficiency in amplification, but may alternatively be double stranded. If double stranded, the primer is first treated to separate its strands before being used to prepare extension products. Preferably, the primer is an oligodeoxyribonucleotide. The primer must be sufficiently long to prime the synthesis of extension products in the presence of the agent for polymerization. The exact lengths of the primers will depend on many factors, including temperature and source of the primer and use of the method. For

example, depending on the complexity of the target sequence, the oligonucleotide primer typically contains about 15–45 nucleotides, although it may contain more or fewer nucleotides. Short primer molecules generally require cooler temperatures to form sufficiently stable hybrid complexes with the template.

The primers used herein are selected to be "substantially" complementary to the different strands of each specific sequence to be amplified. Therefore, the primers need not reflect the exact sequence of the template, but must be sufficiently complementary to selectively hybridize with their respective strands. For example, a non-complementary nucleotide fragment may be attached to the 5′-end of the primer, with the remainder of the primer sequence being complementary to the strand. Alternatively, non-complementary bases or longer sequences can be interspersed into the primer, provided that the primer has sufficient complementarity with the sequence of one of the strands to be amplified to hybridize therewith, and to thereby form a duplex structure which can be extended by the polymerizing means. The non-complementary nucleotide sequences of the primers may include restriction enzyme sites. Appending a restriction enzyme site to the end(s) of the target sequence would be particularly helpful for cloning of the target sequence.

It will be understood that "primer", as used herein, may refer to more than one primer, particularly in the case where there is some ambiguity in the information regarding the terminal sequence(s) of the target region to be amplified. Hence, a "primer" includes a collection of primer oligonucleotides containing sequences representing the possible variations in the sequence or includes nucleotides which allow a typical basepairing.

The oligonucleotide primers may be prepared by any suitable method. Methods for preparing oligonucleotides of specific sequence are known in the art, and include, for example, cloning and restriction of appropriate sequences, and direct chemical synthesis. Chemical synthesis methods may include, for example, the phosphotriester method described by Narang et al. (1979), the phosphodiester method disclosed by Brown et al. (1979), the diethylphosphoramidate method disclosed in Beaucage et al. (1981), and the solid support method in U.S. Pat. No. 4,458,066. The primers may be labeled, if desired, by incorporating means detectable by spectroscopic, photochemical, biochemical, immunochemical, or

chemical means.

Template-dependent extension of the oligonucleotide primer(s) is catalyzed by a polymerizing agent in the presence of adequate amounts of the four deoxyribonucleotide triphosphates (dATP, dGTP, dCTP and dTTP) or analogs, in a reaction medium which is comprised of the appropriate salts, metal cations, and pH buffering system. Suitable polymerizing agents are enzymes known to catalyze primer- and template-dependent DNA synthesis. Known DNA polymerases include, for example, *E. coli* DNA polymerase I or its Klenow fragment, T_4 DNA polymerase, and Taq DNA polymerase. The reaction conditions for catalyzing DNA synthesis with these DNA polymerases are known in the art.

The products of the synthesis are duplex molecules consisting of the template strands and the primer extension strands, which include the target sequence. These products, in turn, serve as template for another round of replication. In the second round of replication, the primer extension strand of the first cycle is annealed with its complementary primer; synthesis yields a "short" product which is bounded on both the 5′- and the 3′-ends by primer sequences or their complements. Repeated cycles of denaturation, primer annealing, and extension result in the exponential accumulation of the target region defined by the primers. Sufficient cycles are run to achieve the desired amount of polynucleotide containing the target region of nucleic acid. The desired amount may vary, and is determined by the function which the product polynucleotide is to serve.

The PCR method can be performed in a number of temporal sequences. For example, it can be performed step-wise, where after each step new reagents are added, or in a fashion where all of the reagents are added simultaneously, or in a partial step-wise fashion, where fresh reagents are added after a given number of steps.

In a preferred method, the PCR reaction is carried out as an automated process which utilizes a thermostable enzyme. In this process the reaction mixture is cycled through a denaturing region, a primer annealing region, and a reaction region. A machine may be employed which is specifically adapted for use with a thermostable enzyme, which utilizes temperature cycling without a liquid handling system, since the

enzyme need not be added at every cycle. This type of machine is commercially available from Perkin Elmer Cetus Corp.

After amplification by PCR, the target polynucleotides are detected by hybridization with a probe polynucleotide which forms a stable hybrid with that of the target sequence under stringent to moderately stringent hybridization and wash conditions. If it is expected that the probes will be completely complementary (i.e., about 99% or greater) to the target sequence, stringent conditions will be used. If some mismatching is expected, for example if variant strains are expected with the result that the probe will not be completely complementary, the stringency of hybridization may be lessened. However, conditions are chosen which rule out nonspecific/adventitious binding. Conditions which affect hybridization, and which select against nonspecific binding are known in the art, and are described in, for example, Maniatis et al. (1982). Generally, lower salt concentration and higher temperature increase the stringency of binding. For example, it is usually considered that stringent conditions are incubation in solutions which contain approximately 0.1×SSC, 0.1% SDS, at about 65° C. incubation/wash temperature, and moderately stringent conditions are incubation in solutions which contain approximately 1–2×SSC, 0.1% SDS and about 50°–65° C. incubation/wash temperature. Low stringency conditions are 2×SSC and about 30°-50°C.

Probes for plasmid target sequences may be derived from well known restriction sites. The plasmid probes may be of any suitable length which span the target region, but which exclude the primers, and which allow specific hybridization to the target region. If there is to be complete complementarity, i.e., if the strain contains a sequence identical to that of the probe, since the duplex will be relatively stable under even stringent conditions, the probes may be short, i.e., in the range of about 10–30 base pairs. If some degree of mismatch is expected with the probe, i.e., if it is suspected that the probe will hybridize to a variant region, the probe may be of greater length, since length seems to counterbalance some of the effect of the mismatch(es).

The probe nucleic acid having a sequence complementary to the target sequence may be synthesized using similar techniques described supra. for the

synthesis of primer sequences. If desired, the probe may be labeled. Appropriate labels are described supra.

In some cases, it may be desirable to determine the length of the PCR product detected by the probe. This may be particularly true if it is suspected that variant plasmid products may contain deletions within the target region, or if one wishes to confirm the length of the PCR product. In such cases it is preferable to subject the products to size analysis as well as hybridization with the probe. Methods for determining the size of nucleic acids are known in the art, and include, for example, gel electrophoresis, sedimentation in gradients, and gel exclusion chromatography.

The presence of the target sequence in a biological sample is detected by determining whether a hybrid has been formed between the polynucleotide probe and the nucleic acid subjected to the PCR amplification technique. Methods to detect hybrids formed between a probe and a nucleic acid sequence are known in the art. For example, for convenience, an unlabeled sample may be transferred to a solid matrix to which it binds, and the bound sample subjected to conditions which allow specific hybridization with a labeled probe; the solid matrix is than examined for the presence of the labeled probe. Alternatively, if the sample is labeled, the unlabeled probe is bound to the matrix, and after the exposure to the appropriate hybridization conditions, the matrix is examined for the presence of label. Other suitable hybridization assays are described supra. Analysis of the nucleotide sequence of the target region(s) may be by direct analysis of the PCR amplified products. A process for direct sequence analysis of PCR amplified products is described in Saiki et al. (1988).

Alternatively, the amplified target sequence(s) may be cloned prior to sequence analysis. A method for the direct cloning and sequence analysis of enzymatically amplified genomic segments has been described by Scharf (1986). In the method, the primers used in the PCR technique are modified near their 5′-ends to produce convenient restriction sites for cloning directly into, for example, an M13 sequencing vector. After amplification, the PCR products are cleaved with the appropriate restriction enzymes. The restriction fragments are ligated into the M13 vector, and transformed into, for example, a JM 103 host, plated out, and the resulting

plaques are screened by hybridization with a labeled oligonucleotide probe. Other methods for cloning and sequence analysis are known in the art.

Construction of the HCV/SEAP reporter gene plasmid

General Method

In the first embodiment, the Tropix® pCMV/SEAP expression vector is used as a starting point for construction of the HCV NS3 protease plasmid construct pHCAP1 (Seq. ID. NOS. 1-7). pHCAP1 is constructed from the pTM3 vector (Moss et al., *Nature*, 348:91-92 (1990)) in which the nucleotide sequence encoding the portion of the HCV-BK polyprotein domains NS2-NS3-NS4A-NS4B was cloned from the pBKCMV/NS2-NS3-NS4A-NS4B-SEAP (the pBK/HCAP) construct. pBK/HCAP is the eukaryotic expression plasmid in which all the original subcloning and ligation of all the HCV NS gene fragments and SEAP gene was created in. pCMV/SEAP is a mammalian expression vector designed for studies of promoter/enhancer elements with SEAP as a reporter (Berger et al., (1988)). The vector contains a polylinker for promoter/enhancer insertion, as well as an intron and polyadenylation signals from SV40. The vector can be propagated in *E.*coli due to the pUC19 derived origin of replication and ampicillin resistance gene. Modification of the commercially available plasmids is accomplished by use of PCR techniques including mutational PCR. Although this particular plasmid is described in the examples that follow, it is not the only plasmid or vector which may be used. The T7 RNA polymerase promoter is part of the pTM3 plasmid which was preferred in construction of the pHCAP vector.

In an alternate embodiment, the pTKgptF2s plasmid (Falkner and Moss, *J. Virol.* 62:1849-1854 (1988)) can be used instead of the pTM3 plasmid, which places the HCV/SEAP gene construct under transcriptional control of the native vaccinia virus promoter. The only requirement is that the promoter operate when placed in a plasmid having vaccinia virus regions flanking the subcloning region. This requirement allows the plasmid homologous recombination with the wild type vaccinia virus. Other vaccinia virus intermediate plasmids would be operable here as well.

Example 1

The Tropix® pCMV/SEAP expression vector is first modified so that both Sac1

restriction sites are inactivated. This is done by cleaving the plasmid with BamH1 which results in a 5' cleavage product that contains the plasmid 5' ATG site and about 250 bp ending at the Bam H1 site, and a 3' cleavage product having BamH1 sites at its 5' end and at its 3' end. The 5' cleavage fragment was then amplified from the pCMV/SEAP plasmid using primers that were designed to delete the 5' ATG codon and to create a Sac 1 site on the 5' end. The downstream 3' primer spanned the Bam H1 site that is present within the SEAP coding sequence. Thus after PCR, the amplified 5' fragment has a 5' Sac 1 site and a Bam H1 site. The 5' primer introduced an extra codon (a glutamic acid residue) in front of the first leucine residue of the SEAP secretion signal. Furthermore, the first leucine codon was changed from a CTG to a CTC codon (a silent change). The codon change was made to create the second half of the Sac 1 site:

5'-GAGCTC-X-GGATCC-3' (Seq. ID NO:22)
Sac 1 site 5' end of SEAP Bam H1

The modified sequence is then cloned into pGEM3Zf(+) (Promega). The Bam H1-Bam H1 SEAP fragment was subcloned into pAlter-1 (Promega) which is a plasmid that has an f1 origin of replication so it produces a single strand DNA for use in oligo mediated site directed mutagenesis. The Sac 1 sites within the SEAP fragment were mutated by oligo mediated site directed mutagenesis (GAGCT<u>C</u> to GAGCT<u>G</u> – a silent change) and the same change at the second Sac 1 site (GAGCT<u>C</u> to GAGCT<u>G</u> – an amino acid change from Serine to Cysteine) The complete SEAP pGEM3Zf(+) plasmid is then made by subcloning the PCR modified 5' SEAP fragment into the Sac I- Bam H1 sites of pGEM3Zf(+). The resulting plasmid was then linearized with Bam H1 to allow the subcloning of the 3' SEAP Bam H1-Bam H1 from the pAlter-1 plasmid which was used for the oligo mediated site directed mutagenesis to disrupt the two internal Sac I sites. A clone with the correct orientation of the Bam H1- Bam H1 fragment distal to the 5' SEAP fragment was selected after of purified plasmid DNA by restriction enzyme digest. This clone was used in the subsequent subcloning steps for the construction of the HCV/SEAP construct.

The coding sequences for the HCV proteins and NS3 cleavage sites that comprise the final HCV/SEAP polyprotein were generated in two separate PCRs from

cDNA of the HCV-BK strain (Accession No. M58335). Takamizawa, A., et al., J. Virol. 65:1105-1113 1991. The first amplified fragment starts with the amino acid coding sequence of the HCV polyprotein corresponding to the C-terminal 81 amino acids of the putative E2 region, which are upstream of the beginning of the putative NS2 region or amino acid 729

(ARVCACLWMMLLIAQAEAALENLVVLNSASVAGAHGILSFLVFFCAAWYIKGRLVPG ATYALYGVWPLLLLLLALPPRAYAMDREMAA) (Seq. ID NO:23)

or nucleotide 2187

(GCACGTGTCTGTGCCTGCTTGTGGATGATGCTGCTGATAGCCCAGGCCGAGGC CGCCTTGGAGAACCTGGTGGTCCTCAATGCGGCGTCTGTGGCCGGCGCACATG GCATCCTCTCCTTCCTTGTGTTCTTCTGTGCCGCCTGGTACATCAAAGGCAGGCT GGTCCCTGGGGCGGCATATGCTCTTTATGGCGTGTGGCCGCTGCTCCTGCTCTT GCTGGCATTACCACCGCGAGCTTACGCCATGGACCGGGAGATGGC) (Seq. ID NO:24)

and contains the DNA encoding the HCV polyprotein domains NS2-NS3-NS4A through the first 176 amino acids of the NS4B gene

(CASHLPYIEQ GMQLAEQFKQ KALGLLQTAT KQAEAAAPVV ESKWRALETF WAKHMWNFIS GIQYLAGLST LPGNPAIASL MAFTASITSPLTTQSTLLFN ILGGWVAAQL APPSAASAFV GAGIAGAAVG SIGLGKVLVD ILAGYGAGVAGALVAFKVMS GEMPSTEDLV NLLPAIL) (Seq. ID NO:25)

or amino acid 1886 or nucleotide 5658

(TGCGCCTCGCACCTCCCTTACATCGAGCAGGGAATGCAGCTCGCCGAGCAATT CAAGCAGAAAGCGCTCGGGTTACTGCAAACAGCCACCAAACAAGCGGAGGCTG CTGCTCCCGTGGTGGAGTCCAAGTGGCGAGCCCTTGAGACATTCTGGGCGAAG CACATGTGGAATTTCATCAGCGGGATACAGTACTTAGCAGGCTTATCCACTCTGC CTGGGAACCCCGCAATAGCATCATTGATGGCATTCACAGCCTCTATCACCAGCC CGCTCACCACCCAAAGTACCCTCCTGTTTAACATCTTGGGGGGGTGGGTGGCTG

CCCAACTCGCCCCCCCCAGCGCCGCTTCGGCTTTCGTGGGCGCCGGCATCGCC GGTGCGGCTGTTGGCAGCATAGGCCTTGGGAAGGTGCTTGTGGACATTCTGGC GGGTTATGGAGCAGGAGTGGCCGGCGCGCTCGTGGCCTTTAAGGTCATGAGCG GCGAGATGCCCTCCACCGAGGACCTGGTCAATCTACTTCCTGCCATC) (Seq. ID NO:26)

The primers used to amplify the fragment were designed to contain an Eco RI site and an ATG codon in the 5' primer (Seq. ID NO:27) and an Xho I site in the 3' primer (Seq. ID NO:28). The amplified fragment was accordingly subcloned as an Eco RI - Xho I fragment into pET24a(+) plasmid (Novagen). The second fragment amplified from the HCV strain BK cDNA encompasses the putative NS5A/5B cleavage site (EEASEDVVCCSMSYTWTGAL)(Seq. ID NO:29). The 5' primer that was used to amplify the cleavage site was designed to have an Xho I site (Seq. ID NO:30) whereas the 3' primer was designed to have a Sac I site (Seq. ID NO:31). The resulting PCR product was subcloned as an Xho I - Sac I fragment into pET24a(+), which had been digested with Xho I- Hind III, as part of a three way ligation (Seq. ID NO:32). The third fragment in the three way ligation was the Sac I - Hind III fragment from the SEAP pGEM3Zf(+) plasmid. The Sac I - Hind III fragment encompassed the modified SEAP gene and also 30 base pairs of the pGEM3Zf(+) polylinker which included the multiple cloning sites (MCS) between the Bam H1 and HindIII sites. The final HCV/SEAP construct was assembled using pBKCMV as the vector. pBKCMV was digested with Eco RI and Hind III and then used in a three way ligation with the NS5A/5B - SEAP Xho I -Hind III fragment and the Eco RI-Xho I NS2-NS4B fragment.

The control plasmids for the assay (pHCAP3, pHCAP4) were constructed in a similar manner to the HCV/SEAP construct. The control plasmids have either an inactive form of NS3 protease or inactive forms of both NS2 protease and NS3 protease. Inactivation of NS2 and NS3 proteases was accomplished by oligo mediated site directed mutagenesis performed on the PCR amplified NS2 - NS4B fragment that had been subcloned into pALTER-1 as an Eco R1 - Xho 1 fragment together with the NS5A/5B Xho 1 - Sac 1 fragment. In order to inactivate the NS3 protease, the catalytic serine residue was substituted with an alanine by replacing thymidine (<u>T</u>CG) with guanine (<u>G</u>CG)(base 2754). The NS2 protease was inactivated by substitution of the catalytic cysteine residue with an alanine residue (<u>TG</u>T -> <u>GC</u>T)(bases 2238-2239). The resulting inactivated NS3 protease and inactivated

NS2-NS3 proteases variants of the NS2-NS4B fragment were each subcloned into pBKCMV as separate Eco R1 - Xho 1 fragments together with the NS5A/5B - SEAP Xho 1 - Hind III fragment.

The pHCAP1 ($NS2^{WT}NS3^{WT}$)(Seq. ID NOS:1-7), pHCAP3 ($NS2^{WT}NS3^{MUT}$)(Seq. ID NOS:8-14), and pHCAP4 ($NS2^{MUT}NS3^{MUT}$) (Seq. ID NOS:15-21) plasmids were constructed using pTM3 as the vector and the appropriate HCV/SEAP fragment from the corresponding pBKHCV/SEAP constructs. The pBKHCV/SEAP constructs were first digested with Eco R1 and the Eco R1 site was filled in using Klenow fragment in a standard fill in reaction. The pBKHCV/SEAP constructs were then digested with Xba I and the gel purified HCV/SEAP fragment was subcloned into pTM3 that had been digested with Sma 1 and Spe 1. Subcloning the HCV/SEAP fragment into the Sma I site will result in an additional 6 amino acids (MGIPQF) (Seq. ID NO:33) at the N-terminus (codons 1426-1444) if the preferred translational start codon, which is part of the Nco 1 site in pTM3, is used.

The pHCAP1 ($NS2^{WT}NS3^{WT}$), pHCAP3 ($NS2^{WT}NS3^{MUT}$), and pHCAP4 ($NS2^{MUT}NS3^{MUT}$) plasmids have been used to generate recombinant vaccinia viruses as described in the next section.

Construction of the HCV/SEAP reporter gene viral vectors

Applicants have generated recombinant vaccinia virus using pHCAP1 and the control plasmids, pHCAP3 and pHCAP4. Recombinant vaccinia viruses were generated using standard procedures in which BSC-1 cells were infected with wild type vaccinia virus (strain WR from ATCC) and then transfected with either pHCAP1, pHCAP3, or pHCAP4. Selection of recombinant virus was performed by growth of infected transfected cells in the presence of mycophenolic acid. The recombinant vaccinia viruses are termed vHCAP1, vHCAP3, and vHCAP4 and correspond directly with the pHCAP1, pHCAP3, and pHCAP4 plasmids. Large scale stocks of the vHCAP1, vHCAP3, and vHCAP4 were grown and titered in CV1 cells.

Transfection of Cell Lines Containing the HCV/SEAP reporter

In the first embodiment HeLa cells are transfected with the Hep C/SEAP reporter gene plasmid, pHCAP1, and co-infection with a vTF7.3, a recombinant vaccinia virus (Fuerst et al., *Proc. Nat. Acad. Sci. USA*, 86:8122-8126 (1986)). vTF7.3 expresses T7 RNA polymerase which is required for transcription of the reporter gene since it is under the control of T7 promoter in the pTM3 plasmid. The pTM3 plasmid is a vaccinia intermediate plasmid which can function as an expression vector in cells when T7 RNA polymerase is provided in *trans* (Figure 2).

As described previously, the Hep C/SEAP reporter gene encodes for a polyprotein with the following gene order: HCV (strain BK) NS2-NS3-NS4A-NS4B' - NS5A/5B cleavage site - SEAP. Thus the HCV sequences for the amino acid coding sequence of the HCV polyprotein corresponding to the C-terminal 81 amino acids of the putative E2 region, which are upstream of the start of the putative NS2 region (as defined by Grakoui et al.) or amino acid 729 and continues through the first 176 amino acids of the NS4B gene or amino acid 1886 (Seq. ID NOS:23-26), and is proximal to the SEAP protein (see Figure 1). The NS5A/5B cleavage site has been engineered between the end of NS4B' and the second codon of SEAP.

The working theory behind the unique design of the reporter gene construct is that the SEAP polyprotein is tethered, as part of the NS2-NS3-NS4A-NS4B' - NS5A/5B cleavage site – SEAP polyprotein, inside the cell. It has been shown that NS2 is a hydrophobic protein and is associated with the outside of the endoplasmic reticulum (ER). Grakoui, et al. (1993). Thus, in the present invention, SEAP is tethered to the ER via the action of NS2. Release of SEAP from the polyprotein tether will occur upon NS3-mediated cleavage at the NS5A/5B cleavage site. SEAP is then secreted from the cell and can be monitored by assaying media for alkaline phosphatase activity (Figure 1B). It is assumed that it is NS3-mediated cleavage at the NS5A/5B site which is the necessary cleavage to release SEAP from the upstream polyprotein sequences. However NS3-mediated cleavage at other sites within the polyprotein may be responsible for SEAP release and hence its subsequent secretion. Both NS3 and NS3/NS4A, where NS4A is a cofactor for NS3, can mediate cleavage at the NS3/4A and NS4A/4B cleavage sites which are present in polyprotein in addition to the engineered NS5A/5B cleavage site. Thus there may be more than

one NS3-mediated cleavage event occurring over the length of the polyprotein before SEAP is available to the cell secretion apparatus and secreted from the cell. Further, in an alternative embodiments the tether may be changed depending upon the chosen cleavage site. In addition, NS2 is an autocatalytic protease; it mediates the cleavage event between it's carboxy-terminal end and the NS3 N-terminus. In the Hep C/SEAP polyprotein, NS2-mediated cleavage at the NS2/NS3 site would release the NS3-NS4A-NS4B'-SEAP polyprotein from the ER.

The above described system can be used to evaluate potent NS3 inhibitors by monitoring the effect of increasing drug concentration on SEAP activity. NS3 inhibition would be detected as a decrease in SEAP activity. Recognizing that a decrease in SEAP activity could also be due to cell cytotoxicity of a given compound or a non-specific effect on vaccinia virus which would adversely effect SEAP transcription, appropriate controls are used as discussed below.

In an alternate embodiment, a "cis-only" cleavage assay is contemplated. In this assay the $NS2^{MUT}NS3^{WT}$ variant of the HCV/SEAP (HCAP2) is used so the polyprotein remains tethered to the outside of the endoplasmic reticulum because the NS2 protease cannot catalyze the cleavage between the C-terminus and the NS3 N-terminus. Thus the only way for SEAP to be released from the tether is if the NS3 protease clips in cis at the NS5A/5B cleavage site. There should not be any trans NS3 mediated cleavage events occurring since NS2 is not available to release the NS3 N-terminus from its tether. The control plasmid or virus for this assay is the $NS2^{MUT}NS3^{MUT}$ variant HCAP4.

DI/DR Assay

A preferred embodiment involves the co-infection of BHK (ATCC No. CCL-10) or CV1 cells (a COS1 derived line ATCC No. CCL-70) cells with both vHCAP1 and vTF7.3 (ATCC No, VR-2153), with CV1 being more preferred. The latter virus is necessary since the Hep C/SEAP gene remains under control of the T7 RNA polymerase promoter in the vHCAP recombinant viruses. Currently both embodiments which are termed the Hep C/SEAP transfection/infection assay, and the dual recombinant vaccinia virus assay (DI/DR assay) respectively, are useful for HCV protease candidate compound evaluation (Figure 3).

Example 1

Protocol for vTF7.3 infection / HCV/SEAP Plasmid Transfection Experiment

Day 1

Flat-bottom 96 well plates were seeded with BHK cells at a density of 1 x 10^4 cells/well (equivalent to about 85% confluence) after 24 hours. In general, one 96 well plate was used for investigation of each compound of interest (protease inhibitor), plus an additional plate at the same cell density is used where two rows are designated for each compound of interest at increasing concentrations for investigating the cytotoxicity of the compounds themselves in cells alone. Cytotoxicity was determined by XTT assay (Sigma 4626).

Day 2

The established monolayer was transfected with either pHCAP1, pHCAP3, pHCAP4, or pTM3 plasmids at a concentration of 0.4 µg/well as part of a DNA Lipofectamine (Gibco BRL) transfection mixture. Infections of the established monolayer with vTF7.3 preceded the transfection step. A working stock of vTF7.3 was diluted to a multiplicity of infection (MOI) of 10 with Optimem. The media was aspirated from the wells (2B-10G) 2 rows at a time. A 50 L aliquot of vTF7.3 inoculum was added per well and gently shaken every 10 minutes. 30 minutes after inoculum addition, the transfection mixes were made by adding 1 mL of Optimem in 3 mL polystyrene tubes. To the media, 48 µg of plasmid DNA was then added to the tubes and mixed, followed by 144 µL of Lipofectamine™, and then the mixture was incubated (R.T.) for 30 minutes. After incubation, 11 mL of Optimem were added to each of the tubes and gently mixed. The vTF7.3 inoculum was aspirated from the wells and 0.1 mL of transfection mix was added to each well and incubated at 34 °C for 4 hours. Compounds/drugs of interest for testing protease inhibition were prepared as stock solutions of 40 mM in 100% DMSO. For assay use, the compounds were diluted to 640 µM (2X) in Optimem with 4% FBS. The compound dilutions were set up in an unused 96 well plate by adding 100 µL Optimem with 4% FBS to wells 4-10 and 150 µL of compound dilutions to all wells in column 3. A serial dilution of the compounds was then performed by transferring 46 µL from well to well across the plate. The transfection mixture was then aspirated from the cells. Then 75

µL of Optimem with 4% FBS was added to the transfected monolayers. Add 75 µL of the 2X compound dilutions to the transfected monolayers and incubated at 34 °C for 48 hours. The cells were checked microscopically at 24 hours and media is collected at 48 hours for measurement of SEAP activity.

SEAP Activity Measurement

After 48 hours, SEAP activity was measured by first transferring 100 µl of media from each well of the 96 well assay plate to a new sterile 96 well plate. Plate(s) were sealed and heated in a heating block at 65 C for 30 minutes. After 30 minutes, plate(s) were removed and cooled to room temperature. For each heat treated plate, we transferred 50 µl of heat treated media to a Dynex (Dynex 7416) 96 well plate. To each well was added 50 µl of Tropix assay buffer and incubated at room temperature for 5 minutes, followed by an addition to each well of 50 µl of Tropix reaction buffer/CSPD substrate (Tropix), each was mixed, and incubated for an additional 90 minutes at room temperature. Chemiluminescence was read in the Victor multilabel counter from Wallac, Inc. (model number 1420) as one second counts and data is reported as luminescent units/second.

For Examples 1 and 2:

XTT Cytotoxicity Assay

XTT (Sigma 4626) was dissolved in phosphate buffered saline (PBS) to a final concentration of 1 mg/mL. 5 mL was prepared per plate. To this solution was added 5 mM PMS (n-methyldibenzopyrazine methyl sulfate salt) (Sigma P9625) to a final concentration of 20 µM. 50 µL of the XTT solution was added per well to the plate set up for cytotoxicity. The plates were incubated at 37 C in a 5% CO_2 incubator for about 3.5 hours and then the color change was quantitated by reading absorbance in a V*max* plate reader (Molecular Devices) at 450nm/650 nm. Values were corrected by subtracting media-only background and presented as %viable with the untreated cell control representing 100%.

<u>Example 2</u>

Representative experiment and resulting data using Protocol of Example 1.

Compounds X, Y, and Z were evaluated in the Vaccinia Virus Infection/ Plasmid Transfection assay as outlined in Example 1. BHK cells were seeded into 96 well plates at a density of 1 x 10^4 cells/well and grown overnight to approximately 85% confluency. The SEAP activity was monitored 48 hours post drug addition in cells transfected with either pHCAP1, pHCAP4, pTM3, or no DNA. Concurrently, Compounds X, Y, and Z were evaluated for cell cytotoxicity in a separate dose response assay using XTT to measure cell viability.

For each compound, cells were infected with vTF7.3 followed by the plasmid transfection step. The arrangement of the cells transfected with one of the three plasmids is illustrated in Figure 9.

Results for Compounds X, Y, and Z are shown in Figures 4 A and 4B and Table 1below. In the three graphs, the amount of SEAP activity detected in cells transfected with the pHCAP1 plasmid ranges from 2 to 7-fold above the amount of SEAP detected in cells transfected with the control plasmids, pHCAP4 and pTM3, or cells only. The EC_{50} (μM) value represents the concentration of drug at which a 50% reduction in SEAP activity is observed relative to the amount of SEAP activity detected in the absence of drug. The CC_{50} (μM) value represents the concentration of drug at which a 50% reduction in cell viability is observed relative to cells in the absence of drug. The ratio of EC_{50}/ CC_{50} yields the therapeutic index (TI) which, by convention, should be greater or equal to 10 in order for a compound to be considered as demonstrating antiviral activity.

Table 1

Compound	EC_{50} (μM)	CC_{50} (μM)	Solubility (μM)	TI
X	45	178	= 100	4
Y	>320	112	= 100	-
Z	>320	112	= 100	-

Within the compound dose range that was examined, only an EC_{50} value for Compound X was obtained. However, since the TI value for Compound X was below 10, it was concluded that Compound X does not represent a candidate inhibitor of NS3 protease activity. Compounds Y and Z did not demonstrate any efficacy in this system and, therefore, are not considered potential candidates (Figs. 4A and 4B).

For Examples 3 and 4:

XTT Cytotoxicity Assay

XTT (Sigma 4626) was dissolved in phosphate buffered saline (PBS) to a final concentration of 1 mg/mL. 5 mL were prepared per plate. To this solution was added 5 mM PMS (n-methyldibenzopyrazine methyl sulfate salt) (Sigma P9625) to a final concentration of 20 µM. This XTT substrate solution was diluted with an equal volume of MEM media containing 4% FBS(V/V). A 100µL/well of this final solution was added to the original plate which still contains the cell monolayer and about 50 µL incubation media. The plates were Incubated at 37 C in a 5% CO2 incubator for about 3.5 hours and then the color change was quantitated by reading absorbance in a V*max* plate reader (Molecular Devices) at 450nm/650 nm. Values were corrected by subtracting media-only background and presented as %viable with the untreated cell control representing 100%.

Example 3

Protocol for Dual Infection/Dose Response (DI/DR) Assay

Day 1

Flat-bottom 96-well plates were seeded with CV1 cells at a density of 1×10^5 cells per well in MEM media containing 10% FBS with no Phenol Red. The plate was set up as shown in Figure 5. Media only was placed in all the wells on the edge of the plate and only one compound is evaluated per plate (Fig. 5).

Day 2

Cells were infected with recombinant vaccinia viruses as follows. There should be about 1.5 x 10^5 cells per well after incubation for 24 hours. For every plate needed (a plate for each drug in the experiment) 4 mL of vTF7.3 in MEM with 4% FBS (-) phenol red at a concentration of 2 x 10^6 pfu/mL was prepared, and divided into 2 mL aliquots. Either vHCAP1 or vHCAP3 was added to the vTF7.3 aliquots for a final concentration of vHCAP of 1 x 10^7 pfu/mL. At 75 μL per well, this concentration of virus stock delivers vTF7.3 at an MOI of 1 and vHCAP1 or vHCAP3 at an MOI of 5. The arrangement of the experimental plate is shown in Figure 5.

Drug stock solutions for use in the assay, were made at a concentration of 40 mM in DMSO as in the previous protocol. The 40 mM drug stock solution was diluted to 640 μM in MEM with 4% FBS (-) phenol red to yield a 2X drug working stock solution. Using an empty 96 well plate, the drug dilution series was set up as follows:

100 μL of MEM with 4% FBS (-) phenol red was added to all wells in columns 4-10. 150 μL of 2X drug working stock solution was added to all wells in column 3. 46 μL of media was transferred from column 3 to wells of column 4 and mixed. Transferring of 46 μL from column 4 to column 5 and out to row 10 was repeated. The remaining 46 μL was discarded. The arrangement of the experimental multiwell plate is shown in Figure 6.

Media was aspirated from the CV1 monolayers. After aspiration, 75 μL per well of appropriate virus inoculum or MEM with 4% FBS (-) phenol red was added to the CV1 monolayers, then 75 μL was transferred from each well in the drug dilution series plate to the corresponding wells on the cell monolayer plate. The assay plate was incubated at 37 C in a 5% CO_2 incubator for 48 hours.

At Day 3, the cells was microscopically checked for phenotypic changes around the 24 hour time point. At Day 4, 100 μL of media was collected from each well of which 50 μL was used in the measurement of SEAP activity. The 100 μL aliquots were transferred to an unused 96 well plate and after the plate was sealed, it was heated to 65 C for 30 minutes. 50 μL of each heat treated sample was then transferred to its corresponding well in a new 96 well opaque plate (Dynex 7416). Using the Tropix® SEAP Phosphalight™ kit, 50 mL of Tropix assay buffer was added

to each well and the plate was incubated at room temperature for 5 minutes. Next, 50 μL of Tropix reaction buffer/CPSD substrate was added and mixed. The plate was incubated for 90 minutes at room temperature. The chemiluminescence was then read using a Victor multi-label counter. The XTT assay for measuring cytotoxicity was also performed on Day 4 as described.

Example 4

Representative Experiment and Resulting Data Using Protocol of Example 3

Compounds A -I were evaluated in the DI/ DR assay using the standard protocol given in Example 3. The data shown in Figure 7 and Figure 8 represent assay results obtained at a 48 hour time point post drug addition.

The EC_{50} (μM) value represents the concentration of drug at which a 50% reduction in SEAP activity is observed relative to the amount of SEAP activity detected in the absence of drug. However, this latter value, the amount of SEAP activity that is observed in the absence of drug, is first corrected for assay background prior to the calculation of an EC_{50} value. The correction is made since in the inactive NS3 protease construct, vHCAP3, a background level of SEAP activity is detected (see SEAP Activity graph). This background SEAP activity represents non-NS3 protease mediated SEAP activity and therefore should not be affected by the addition of an NS3 protease inhibitor. It is assumed that a fraction of the SEAP activity that is observed in the active NS3 protease construct, vHCAP1, represents non-NS3 protease mediated SEAP activity. Therefore the amount of SEAP activity detected vHCAP1 is corrected for the fraction that corresponds to non-NS3 protease mediated SEAP activity. The correction is as follows: luminescent units of SEAP activity of vHCAP1 - luminescent units of SEAP activity of vHCAP3 = Value N (level of NS3 protease dependent SEAP activity). Accordingly, (vHCAP1/SEAP)-N/2 = EC_{50} value.

The CC_{50} (μM) value represents the concentration of drug at which a 50% reduction in cell viability is observed relative to cells in the absence of drug. The ratio of EC_{50}/ CC_{50} yields the therapeutic index (TI) which, by convention, should be

greater or equal to 10 in order for a compound to be considered as demonstrating antiviral activity.

In Figure 7, increasing concentrations of Compound A were observed to have no affect on SEAP activity. In the cell cytotoxicity component of the assay, it was observed that increasing concentrations of Compound A did not result in a reduction of cell viability of cells alone or cells infected with either vHCAP1/vTF7.3 or vHCAP3/vTF7.3. The results obtained with Compounds B - I (Figure 8) demonstrate a range of observed cytotoxicities from 15 μM to >320 μM which is the upper limit of drug concentrations tested in the DI/ DR assay although it is theoretically possible to test drug concentrations above 320 μM. The EC_{50} values that were observed for Compounds B - I ranged from 18 μM to > 320 μM, however, the TI values were under 10. Thus Compounds A -I do not represent potential inhibitors of NS3 protease activity.

We Claim:

1. A reporter gene system useful in the assessment of compounds which augment or inhibit the activity of Hepatitis C virus NS3 protease comprising:

a) a recombinant viral vector comprising a DNA molecule encoding an RNA polymerase promoter compatible with said viral vector and which is expressed upon infection of a target mammalian cell;

b) a recombinant plasmid comprising a DNA molecule encoding the HCV/SEAP reporter gene polyprotein which is expressed when transfected into a target mammalian cell;

c) said target mammalian cell line being infected first with said recombinant viral vector then transfected with said recombinant plasmid such that the DNA molecule encoding the HCV/SEAP reporter gene is under transcriptional control of said promoter; and

d) the target mammalian cell expressing said HCV/SEAP reporter gene polyprotein such that SEAP is secreted from said target mammalian cell.

2. A reporter gene system useful in the assessment of compounds which augment or inhibit the activity of Hepatitis C virus NS3 protease comprising:

a) a first recombinant viral vector comprising a DNA molecule encoding an RNA polymerase promoter compatible with said viral vector and which is expressed upon infection of a target mammalian cell;

b) a second recombinant viral vector comprising a DNA molecule encoding the HCV/SEAP reporter gene polyprotein which is expressed upon infection of a target mammalian cell;

c) said target mammalian cell line being infected first with said first

recombinant viral vector then co-infected with said second recombinant plasmid such that the DNA molecule encoding the HCV/SEAP reporter gene is under control of said promoter; and

d) the target mammalian cell expresses said HCV/SEAP reporter gene polyprotein such that SEAP is secreted from said target mammalian cell.

3. The reporter gene system of claim 1 wherein said recombinant plasmid is the pTM3 plasmid containing said HepC/SEAP construct.

4. The recombinant plasmid of claim 3 wherein said recombinant plasmid comprises the pHCAP1 plasmid having a DNA molecule encoding the NS2 and NS3 protease polyproteins in a fusion protein fused with the SEAP gene according to the sequence in Seq. ID NO: 1.

5. The recombinant plasmid of claim 3 wherein said recombinant plasmid further comprises the pHCAP3 plasmid containing the active NS2 protease and a mutant NS3 protease in a fusion protein fused with the SEAP gene according to the sequence in Seq. ID NO: 8.

6. The recombinant plasmid of claim 3 wherein said recombinant plasmid further comprises the pHCAP4 plasmid containing the mutant inactive NS2 and mutant inactive NS3 protease in a fusion protein fused with the SEAP gene according to the sequence in Seq. ID NO: 15.

7. The reporter gene system of claim 2 wherein said second recombinant viral vector further comprises the vHCAP1 vector having a DNA molecule encoding the NS2 and NS3 protease polyproteins in a fusion protein fused with the SEAP gene according to the sequence in Seq. ID NO: 1.

8. The reporter gene system of claim 2 wherein said second recombinant viral vector further comprises the vHCAP3 vector containing the active NS2 protease and a mutant NS3 protease in a fusion protein fused with the SEAP gene according to the sequence in Seq. ID NO: 9.

9. The reporter gene system of claim 2 wherein said second recombinant viral vector further comprises the vHCAP4 vector containing the active NS2 protease and a mutant NS3 protease in a fusion protein fused with the SEAP gene according to the sequence in Seq. ID NO: 16.

10. The reporter gene system of claim 1 wherein said recombinant viral vector comprises a virus containing the DNA sequence encoding T7 RNA polymerase promoter.

11. The recombinant viral vector of claim 7 wherein said vector is the vTF7.3 vector.

12. The reporter gene system of claim 2 wherein said first recombinant viral vector comprises a virus containing the DNA sequence encoding the T7 RNA polymerase promoter.

13. The recombinant viral vector of claim 9 wherein said vector is the vTF7.3 vector.

14. The reporter gene system of claim 1 wherein said first recombinant viral vector comprises a virus containing the DNA sequence encoding a vaccinia virus compatible promoter.

15. The first recombinant viral vector of claim 11 wherein said vector is a vaccinia virus derived vector.

16. The reporter gene system of claim 2 wherein said first recombinant viral vector comprises a virus containing the DNA sequence encoding a vaccinia virus compatible promoter.

17. The first recombinant viral vector of claim 13 wherein said vector is a vaccinia virus derived vector.

18. A first recombinant viral vector according to claim 2 wherein the vector is pTM3 plasmid, a Listeria vector, an orthopox virus, avipox virus, canarypox virus, suipox virus, vaccinia virus, baculovirus, human adenovirus, SV40, Herpes Virus or bovine papilloma virus.

19. A second recombinant viral vector according to claim 2 wherein the vector is pTM3 plasmid, a Listeria vector, an orthopox virus, avipox virus, canarypox virus, suipox virus, vaccinia virus, baculovirus, human adenovirus, SV40, Herpes Virus or bovine papilloma virus.

20. The reporter gene system of claim 1 wherein said recombinant viral vector comprises a virus containing a the DNA sequence encoding a promoter selected from the group of mammalian viral vectors consisting of:

Simian Virus 40 (SV40), Rous Sarcoma Virus (RSV), Adenovirus (ADV) and Bovine Papilloma Virus (BPV).

21. The reporter gene system of claim 2 wherein said recombinant viral vector comprises a virus containing a the DNA sequence encoding a promoter selected from the group of mammalian viral vectors consisting of:

Simian Virus 40 (SV40), Rous Sarcoma Virus (RSV), Adenovirus (ADV) and Bovine Papilloma Virus (BPV).

22. The reporter gene system of claim 1 wherein said target cell line is selected from the group consisting of:

HeLa cells, Chinese Hamster Ovary cells, CV1 African Green Monkey cells, BSC 1 cells and Baby Hamster Kidney cells.

23. The reporter gene system of claim 2 wherein said target cell line is selected from the group consisting of:

HeLa cells, Chinese Hamster Ovary cells, CV1 African Green Monkey cells, BSC 1 cells and Baby Hamster Kidney cells.

24. An isolated DNA sequence comprising a DNA sequence or variants thereof encoding the HepC/SEAP reporter gene construct according to claim 1.

25. The isolated DNA sequence of claim 24 comprising a DNA sequence or variants thereof in SEQ. ID NO. 1.

26. An isolated DNA sequence comprising a DNA sequence or variants thereof encoding the sequence defined as pHCAP1.

27. An isolated DNA sequence comprising a DNA sequence or variants thereof encoding the sequence defined as pHCAP3.

28. An isolated DNA sequence comprising a DNA sequence or variants thereof encoding the sequence defined as pHCAP4.

29. An isolated DNA sequence comprising a DNA sequence or variants thereof encoding the sequence defined as vHCAP1.

30. An isolated DNA sequence comprising a DNA sequence or variants thereof encoding the sequence defined as vHCAP3.

31. An isolated DNA sequence comprising a DNA sequence or variants thereof encoding the sequence defined as vHCAP4.

32. A method of assessing compounds which augment or inhibit the activity of Hepatitis C virus NS3 protease comprising:

a) a control target mammalian cell;

b) a first target mammalian cell expressing the pHCAP1 polyprotein;

c) a second target mammalian cell expressing the pHCAP4 polyprotein;

d) a third target mammalian cell expressing the viral promoter only;

e) incubating said control, first, second, and third target mammalian cells for about 24 hours in a suitable growth medium in the presence and/or absence of pharmacologically effective concentrations of candidate compounds;

f) measuring the amount of SEAP activity; and

g) determining whether said candidate compounds augmented or inhibited hepatitis C NS3 protease by comparing the SEAP activity of said control, first, second, and third target mammalian cells.

33. A method of assessing compounds which augment or inhibit the activity of Hepatitis C virus NS3 protease comprising:

a) a control target mammalian cell;

b) a first target mammalian cell expressing the vHCAP1 polyprotein;

c) a second target mammalian cell expressing the vHCAP4 polyprotein;

d) a third target mammalian cell expressing the viral promoter only;

e) incubating said control, first, second, and third target mammalian cells for about 24 hours in a suitable growth medium in the presence and/or absence of pharmacologically effective concentrations of candidate compounds;

f) measuring the amount of SEAP activity; and

g) determining whether said candidate compounds augmented or inhibited hepatitis C NS3 protease by comparing the SEAP activity of said control, first, second, and third target mammalian cells.

34. A method of assessing compounds which augment or inhibit the activity of Hepatitis C virus NS3 protease cis-only cleavage comprising:

a) a control target mammalian cell;

b) a first target mammalian cell expressing the pHCAP3 polyprotein;

c) a second target mammalian cell expressing the pHCAP4 polyprotein;

d) a third target mammalian cell expressing the viral promoter only;

e) incubating said control, first, second, and third target mammalian cells for about 24 hours in a suitable growth medium in the presence and/or absence of pharmacologically effective concentrations of candidate compounds;

f) measuring the amount of SEAP activity; and

g) determining whether said candidate compounds augmented or inhibited hepatitis C NS3 protease by comparing the SEAP activity of said control, first, second, and third target mammalian cells.

35. A process for constructing a reporter gene system useful in the assessment of compounds which augment or inhibit the activity of Hepatitis C virus NS3 protease comprising:

a) providing a recombinant viral vector comprising a DNA molecule encoding an RNA polymerase promoter compatible with said viral vector and which is expressed upon infection of a target mammalian cell;

b) providing a recombinant plasmid comprising a DNA molecule encoding the HCV/SEAP reporter gene polyprotein which is expressed when transfected into a target mammalian cell further comprising the steps of

cloning into a suitable vector the NS2-NS3-NS4A-NS4B' -NS5A/5B cleavage site – SEAP polyprotein;

c) said target mammalian cell line being infected first with said recombinant viral vector then transfected with said recombinant plasmid such that the DNA molecule encoding the HCV/SEAP reporter gene is under transcriptional control of said promoter; and

d) the target mammalian cell expressing said HCV/SEAP reporter gene polyprotein such that SEAP is secreted from said target mammalian cell.

36. A process for constructing a reporter gene system useful in the assessment of compounds which augment or inhibit the activity of Hepatitis C virus NS3 protease comprising:

a) providing a first recombinant viral vector comprising a DNA molecule encoding an RNA polymerase promoter compatible with said viral vector and which is expressed upon infection of a target mammalian cell;

b) providing a second recombinant viral vector comprising a DNA molecule encoding the HCV/SEAP reporter gene polyprotein which is expressed when transfected into a target mammalian cell further comprising the steps of cloning into a suitable vector the NS2-NS3-NS4A-NS4B' -NS5A/5B cleavage site – SEAP polyprotein;

c) said target mammalian cell line being infected first with said first recombinant viral vector then co-infected with said second recombinant plasmid such that the DNA molecule encoding the HCV/SEAP reporter gene is under control of said promoter; and

d) the target mammalian cell expresses said HCV/SEAP reporter gene polyprotein such that SEAP is secreted from said target mammalian

cell.

37. The isolated DNA sequence of claim 27 comprising a DNA sequence or variants thereof in SEQ. ID NO. 8.

38. The isolated DNA sequence of claim 28 comprising a DNA sequence or variants thereof in SEQ. ID NO. 15.

39. A composition comprising the pHCAP1 polyprotein as described in SEQ. ID NO. 2.

40. A composition comprising the pHCAP3 polyprotein as described in SEQ. ID NO. 9.

41. A composition comprising the pHCAP4 polyprotein as described in SEQ. ID NO. 16.

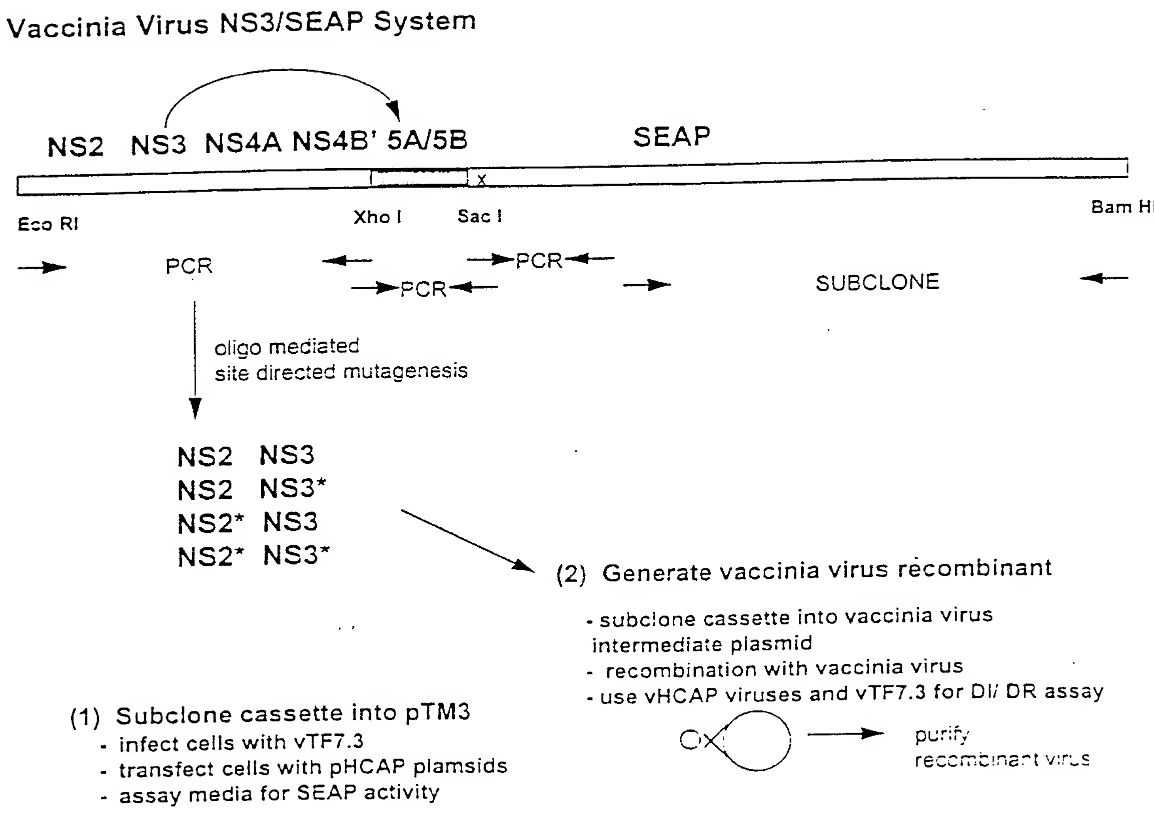

Vaccinia Virus NS3/SEAP System
NS2 NS3 NS4A NS4B' 5A/5B
SEAP
Eco RI
Xho I
Sac I
Bam HI
PCR
PCR
PCR
SUBCLONE
oligo mediated
site directed mutagenesis
NS2 NS3
NS2 NS3*
NS2* NS3
NS2* NS3*
(2) Generate vaccinia virus recombinant
- subclone cassette into vaccinia virus intermediate plasmid
- recombination with vaccinia virus
- use vHCAP viruses and vTF7.3 for DI/ DR assay
purify
recombinant virus
(1) Subclone cassette into pTM3
- infect cells with vTF7.3
- transfect cells with pHCAP plamsids
- assay media for SEAP activity

Figure 1

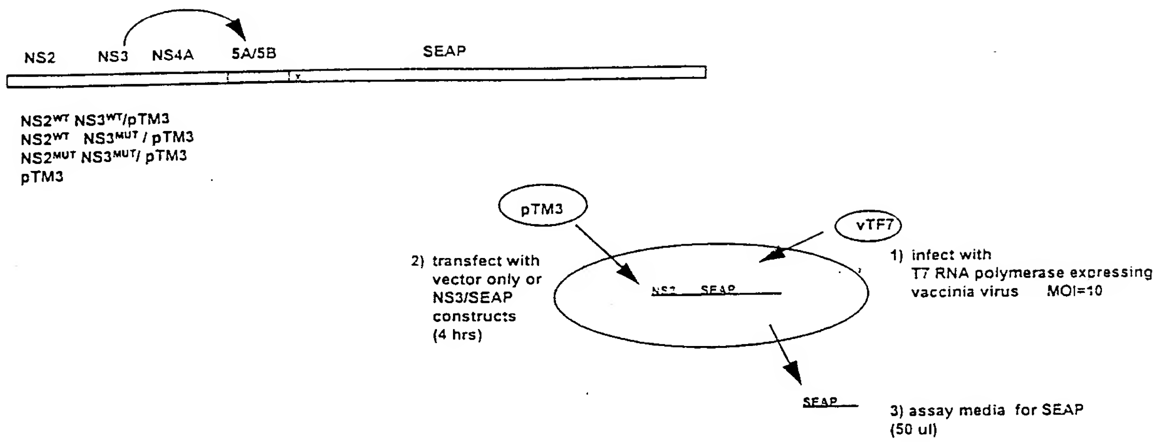

NS2
NS3
NS4A
5A/5B
SEAP
NS2WT NS3WT/pTM3
NS2WT NS3MUT / pTM3
NS2MUT NS3MUT/ pTM3
pTM3
pTM3
vTF7
2) transfect with vector only or NS3/SEAP constructs (4 hrs)
1) infect with T7 RNA polymerase expressing vaccinia virus MOI=10
SEAP
3) assay media for SEAP (50 ul)

Figure 1B

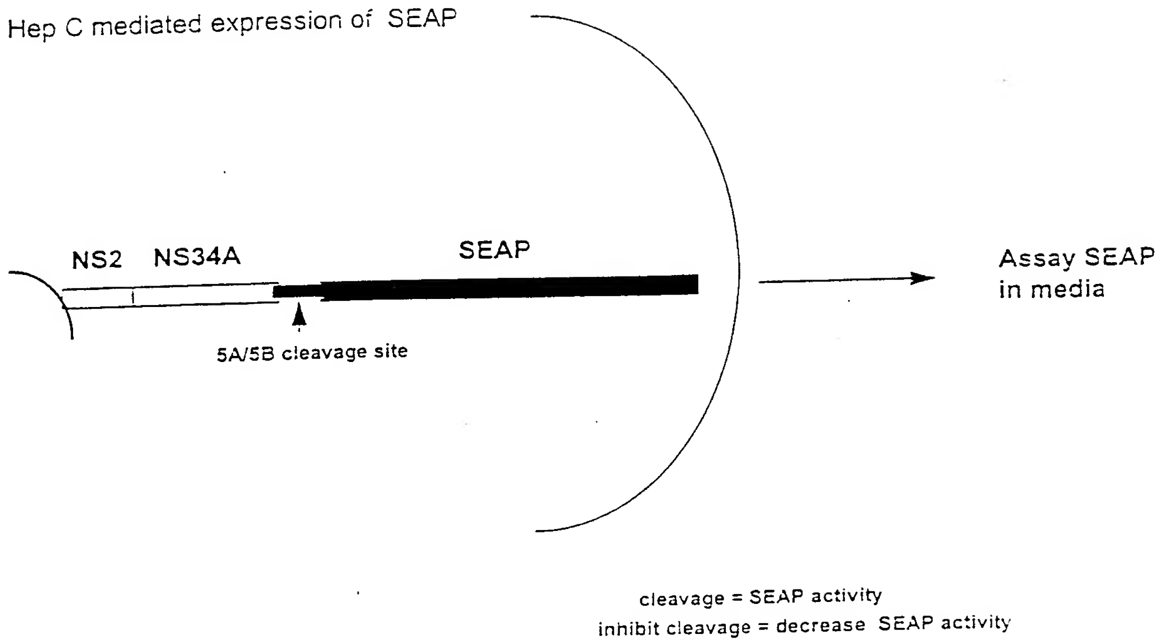

Hep C mediated expression of SEAP
NS2
NS34A
SEAP
5A/5B cleavage site
Assay SEAP in media
cleavage = SEAP activity
inhibit cleavage = decrease SEAP activity

Figure 2

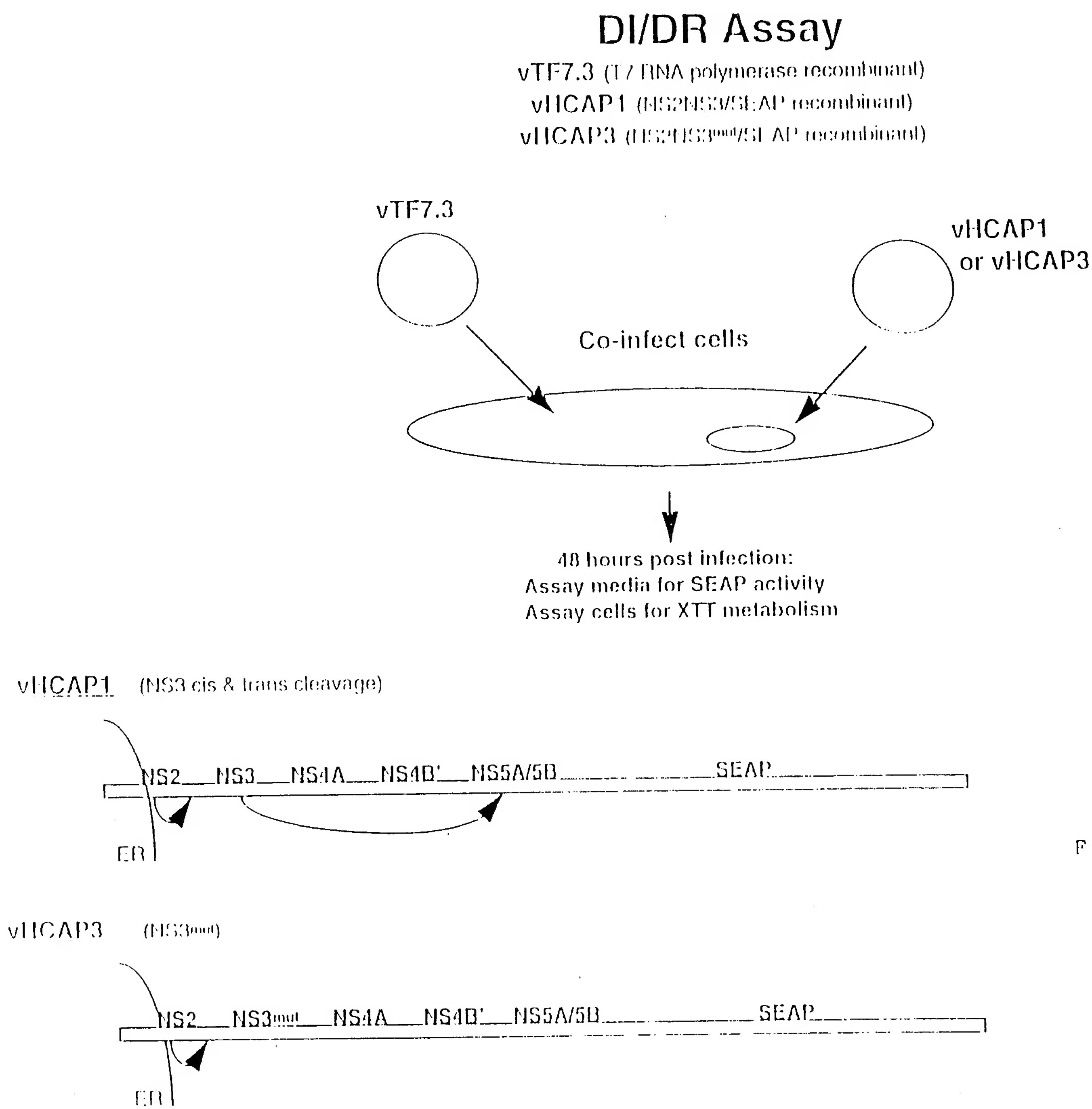
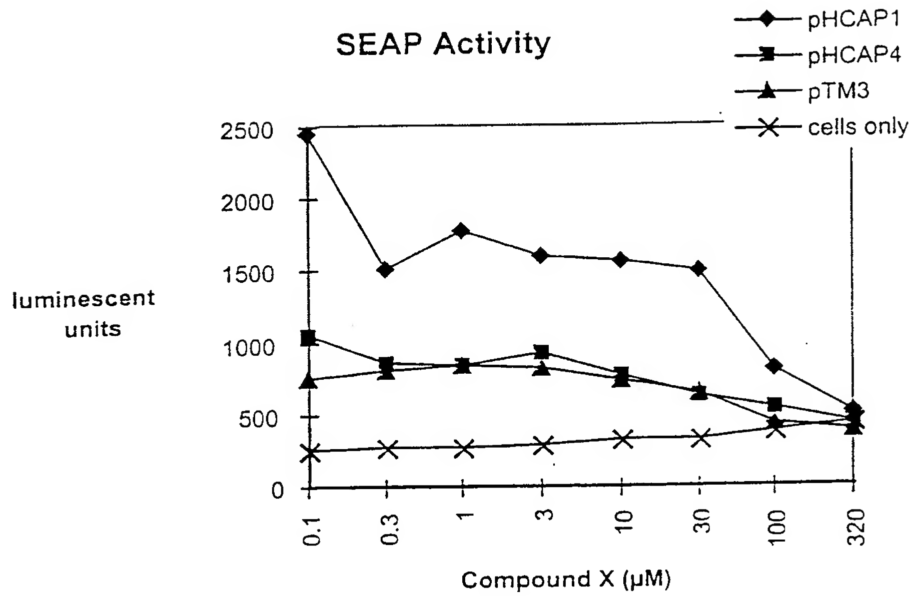

DI/DR Assay
vTF7.3 (T7 RNA polymerase recombinant)
vHCAP1 (NS2NS3/SEAP recombinant)
vHCAP3 (NS2NS3mut/SEAP recombinant)
vTF7.3
vHCAP1
or vHCAP3
Co-infect cells
48 hours post infection:
Assay media for SEAP activity
Assay cells for XTT metabolism
vHCAP1 (NS3 cis & trans cleavage)
NS2 NS3 NS4A NS4B' NS5A/5B SEAP
ER
vHCAP3 (NS3mut)
NS2 NS3mut NS4A NS4B' NS5A/5B SEAP
ER

Figure 3


SEAP Activity
pHCAP1
pHCAP4
pTM3
cells only
luminescent units
2500
2000
1500
1000
500
0
0.1
0.3
1
3
10
30
100
320
Compound X (µM)

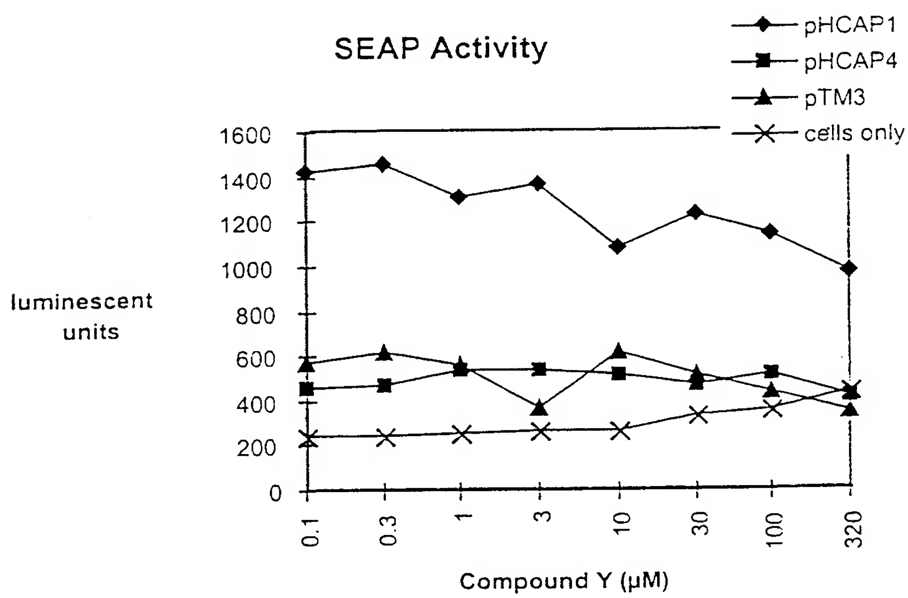

SEAP Activity
pHCAP1
pHCAP4
pTM3
cells only
luminescent units
1600
1400
1200
1000
800
600
400
200
0
0.1
0.3
1
3
10
30
100
320
Compound Y (µM)

Figure 4 A

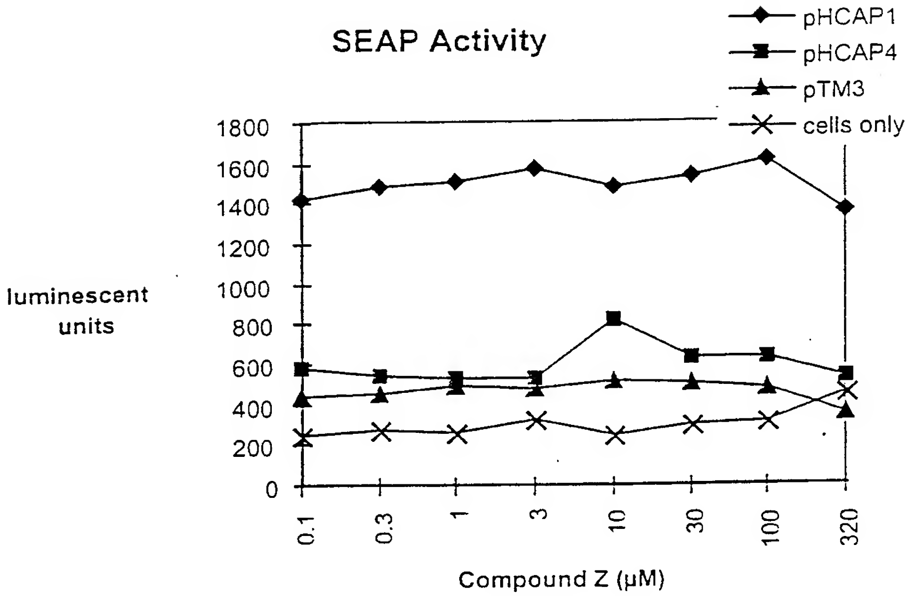

SEAP Activity
pHCAP1
pHCAP4
pTM3
cells only
luminescent units
1800
1600
1400
1200
1000
800
600
400
200
0
0.1
0.3
1
3
10
30
100
320
Compound Z (μM)

Figure 4 B

Figure 5

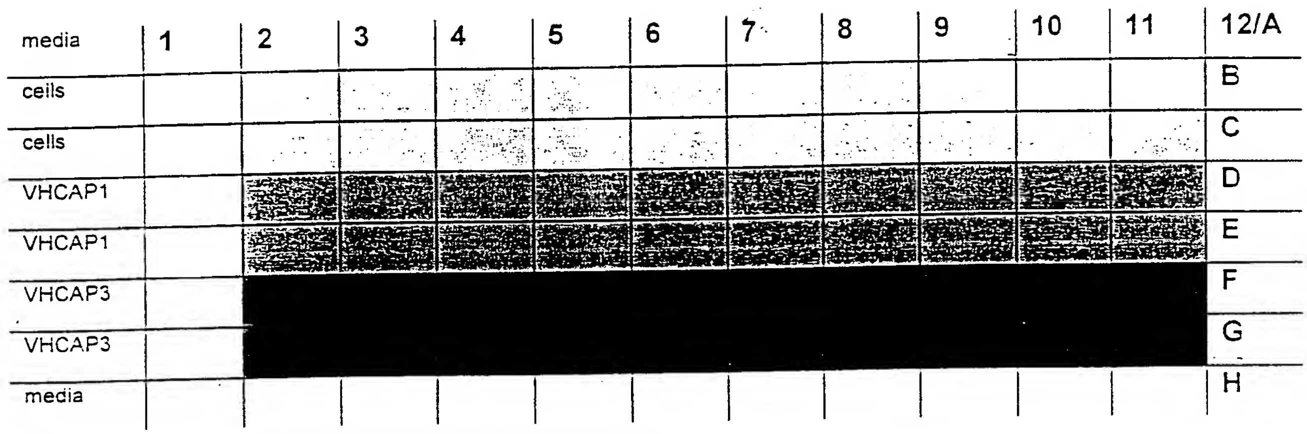

media
1
2
3
4
5
6
7
8
9
10
11
12/A
cells
B
cells
C
VHCAP1
D
VHCAP1
E
VHCAP3
F
VHCAP3
G
media
H

Figure 6.

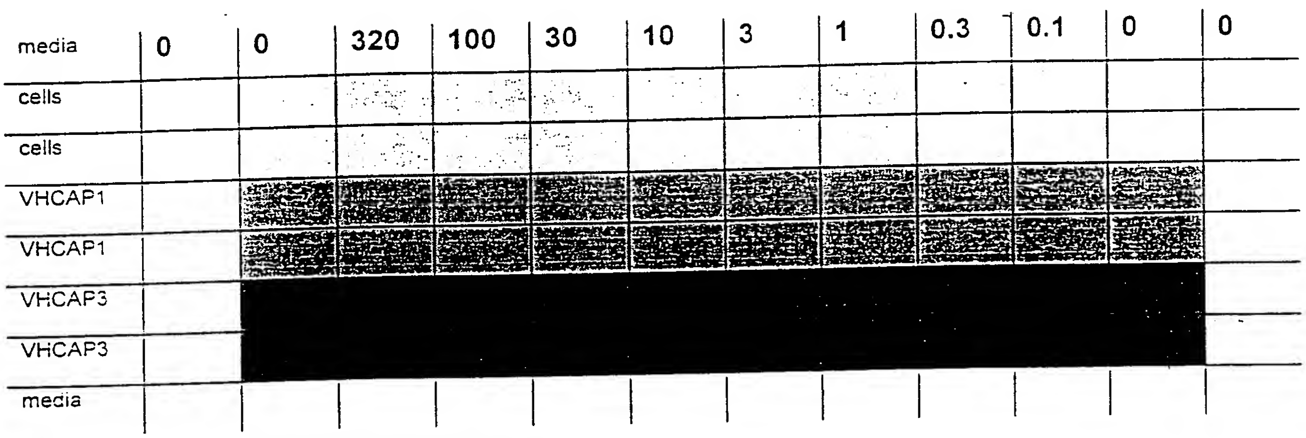
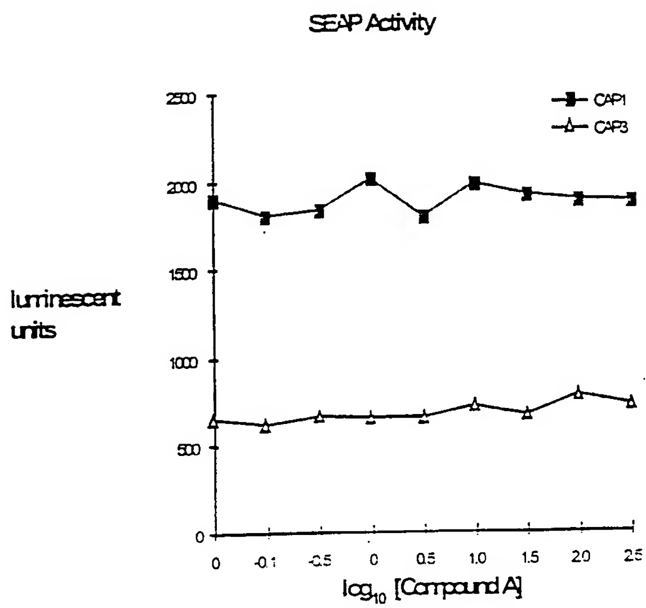

media
0
0
320
100
30
10
3
1
0.3
0.1
0
0
cells
cells
VHCAP1
VHCAP1
VHCAP3
VHCAP3
media

Figure 7


SEAP Activity
CAP1
CAP3
luminescent units
2500
2000
1500
1000
500
0
0
-0.1
-0.5
0
0.5
1.0
1.5
2.0
2.5
$\log_{10}$ [Compound A]

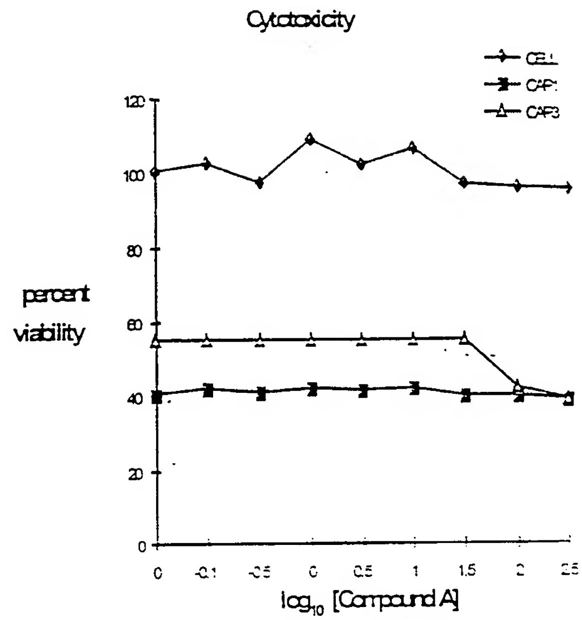

Cytotoxicity
CELL
CAP1
CAP3
percent viability
120
100
80
60
40
20
0
0
-0.1
-0.5
0
0.5
1
1.5
2
2.5
$\log_{10}$ [Compound A]

Figure 8

DI/ DR Assay Compound Summary

Compound	EC_{50} (μM)	CC_{50} (μM)	TI	Solubility	Activity
A	> 320	> 320	-	> 320	-
B	18	15	1	> 320	-
C	37	41	1	> 320	-
D	> 320	> 320	-	> 320	-
E	70	174	2	ppt > 30	-
F	64	> 320	4	> 320	+/ -
G	> 320	> 320	-	> 320	-
H	166	194	1	> 320	-
I.	38	76	2	> 320	-

Plate map:

		1	2	3	4	5	6	7	8	9	10	11	12	
	µM Compound	0•	0•	320•	100•	30•	10•	3•	1•	0.3•	0.1•	0•	0•	Compounds
A	BHK ONLY													W
B	pHCAP1													X
C	pHCAP1													Y
D	pHCAP4													Z
E	pHCAP4													
F	pTM3													
G	pTM3													
H	BHK ONLY													

Figure 9

SEQUENCE LISTING

<110> Potts, Karen E.
Jackson, Roberta L.
Patick, Amy K.

<120> REPORTER GENE SYSTEM FOR USE IN CELL-BASED ASSESSMENT OF INHIBITORS OF THE HEPATITIS C VIRUS PROTEASE

<130> 0125-0005A

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caatatcgca ttttctaacg tgatggatat attaaagtcg aataaagtga acaataatta 480

attctttatt gtcatc atg aac ggc gga cat att cag ttg ata atc ggc ccc 532
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        15                  20                  25

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Gln Ile Ala Gln Tyr Lys Cys Val Thr Ile Lys Tyr Ser Asn Asp Asn
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aga tac gga acg gga cta tgg acg cat gat aag aat aat ttt gaa gca    676
Arg Tyr Gly Thr Gly Leu Trp Thr His Asp Lys Asn Asn Phe Glu Ala
45                  50                  55                  60

ttg gaa gca act aaa cta tgt gat gtc ttg gaa tca att aca gat ttc    724
Leu Glu Ala Thr Lys Leu Cys Asp Val Leu Glu Ser Ile Thr Asp Phe
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Ile Pro Gln Phe Met Ala Arg Val Cys Ala Cys Leu Trp Met Met Leu
 95                 100                 105                 110

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Leu Ile Ala Gln Ala Glu Ala Ala Leu Glu Asn Leu Val Val Leu Asn
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Ala Ala Ser Val Ala Gly Ala His Gly Ile Leu Ser Phe Leu Val Phe
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Phe Cys Ala Ala Trp Tyr Ile Lys Gly Arg Leu Val Pro Gly Ala Ala
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tat gct ctt tat ggc gtg tgg ccg ctg ctc ctg ctc ttg ctg gca tta    1670
Tyr Ala Leu Tyr Gly Val Trp Pro Leu Leu Leu Leu Leu Leu Ala Leu
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cca ccg cga gct tac gcc atg gac cgg gag atg gct gca tcg tgc gga    1718
Pro Pro Arg Ala Tyr Ala Met Asp Arg Glu Met Ala Ala Ser Cys Gly
175                 180                 185                 190

ggc gcg gtt ttt gtg ggt ctg gta ctc ctg act ttg tca cca tac tac    1766
Gly Ala Val Phe Val Gly Leu Val Leu Leu Thr Leu Ser Pro Tyr Tyr
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aag gtg ttc ctc gct agg ctc ata tgg tgg tta caa tat ttt acc acc    1814
Lys Val Phe Leu Ala Arg Leu Ile Trp Trp Leu Gln Tyr Phe Thr Thr
            210                 215                 220

aga gcc gag gcg cac tta cat gtg tgg atc ccc ccc ctc aac gct cgg    1862
Arg Ala Glu Ala His Leu His Val Trp Ile Pro Pro Leu Asn Ala Arg
        225                 230                 235

gga ggc cgc gat gcc atc atc ctc ctc atg tgc gca gtc cat cca gag    1910
Gly Gly Arg Asp Ala Ile Ile Leu Leu Met Cys Ala Val His Pro Glu
    240                 245                 250

cta atc ttt gac atc acc aaa ctt cta att gcc ata ctc ggt ccg ctc    1958
Leu Ile Phe Asp Ile Thr Lys Leu Leu Ile Ala Ile Leu Gly Pro Leu
255                 260                 265                 270
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Gln Gly Leu Ile His Ala Cys Met Leu Val Arg Lys Val Ala Gly Gly
290 295 300

cat tat gtc caa atg gcc ttc atg aag ctg ggc gcg ctg aca ggc acg 2102
His Tyr Val Gln Met Ala Phe Met Lys Leu Gly Ala Leu Thr Gly Thr
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Tyr Ile Tyr Asn His Leu Thr Pro Leu Arg Asp Trp Ala His Ala Gly
320 325 330

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Leu Arg Asp Leu Ala Val Ala Val Glu Pro Val Val Phe Ser Asp Met
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Glu Thr Lys Ile Ile Thr Trp Gly Ala Asp Thr Ala Ala Cys Gly Asp
355 360 365

atc atc ttg ggt ctg ccc gtc tcc gcc cga agg gga aag gag ata ctc 2294
Ile Ile Leu Gly Leu Pro Val Ser Ala Arg Arg Gly Lys Glu Ile Leu
370 375 380

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385 390 395

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Pro Ile Thr Ala Tyr Ser Gln Gln Thr Arg Gly Leu Leu Gly Cys Ile
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Ile Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu Gly Glu Val
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Arg Pro Val Ser Tyr Leu Lys Gly Ser Ser Gly Gly Pro Leu Leu Cys
            530                 535                 540

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Pro Ser Gly His Ala Val Gly Ile Phe Arg Ala Ala Val Cys Thr Arg
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ggg gtt gcg aag gcg gtg gac ttt gtg ccc gta gag tcc atg gaa act    2870
Gly Val Ala Lys Ala Val Asp Phe Val Pro Val Glu Ser Met Glu Thr
    560                 565                 570

act atg cgg tct ccg gtc ttc acg gac aac tca tcc ccc ccg gcc gta    2918
Thr Met Arg Ser Pro Val Phe Thr Asp Asn Ser Ser Pro Pro Ala Val
575                 580                 585                 590

ccg cag tca ttt caa gtg gcc cac cta cac gct ccc act ggc agc ggc    2966
Pro Gln Ser Phe Gln Val Ala His Leu His Ala Pro Thr Gly Ser Gly
                595                 600                 605

aag agt act aaa gtg ccg gct gca tat gca gcc caa ggg tac aag gtg    3014
Lys Ser Thr Lys Val Pro Ala Ala Tyr Ala Ala Gln Gly Tyr Lys Val
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ctc gtc ctc aat ccg tcc gtt gcc gct acc tta ggg ttt ggg gcg tat    3062
Leu Val Leu Asn Pro Ser Val Ala Ala Thr Leu Gly Phe Gly Ala Tyr
        625                 630                 635

atg tct aag gca cac ggt att gac ccc aac atc aga act ggg gta agg    3110
Met Ser Lys Ala His Gly Ile Asp Pro Asn Ile Arg Thr Gly Val Arg
    640                 645                 650

acc att acc aca ggc gcc ccc gtc aca tac tct acc tat ggc aag ttt    3158
Thr Ile Thr Thr Gly Ala Pro Val Thr Tyr Ser Thr Tyr Gly Lys Phe
655                 660                 665                 670

ctt gcc gat ggt ggt tgc tct ggg ggc gct tat gac atc ata ata tgt    3206
Leu Ala Asp Gly Gly Cys Ser Gly Gly Ala Tyr Asp Ile Ile Ile Cys
                675                 680                 685

gat gag tgc cat tca act gac tcg act aca atc ttg ggc atc ggc aca    3254
Asp Glu Cys His Ser Thr Asp Ser Thr Thr Ile Leu Gly Ile Gly Thr
            690                 695                 700

gtc ctg gac caa gcg gag acg gct gga gcg cgg ctt gtc gtg ctc gcc    3302
Val Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Val Val Leu Ala
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acc gct acg cct ccg gga tcg gtc acc gtg cca cac cca aac atc gag    3350
Thr Ala Thr Pro Pro Gly Ser Val Thr Val Pro His Pro Asn Ile Glu
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gag gtg gcc ctg tct aat act gga gag atc ccc ttc tat ggc aaa gcc    3398
Glu Val Ala Leu Ser Asn Thr Gly Glu Ile Pro Phe Tyr Gly Lys Ala
735                 740                 745                 750
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tcc aag aag aag tgc gac gag ctc gcc gca aag ctg tca ggc ctc gga    3494
Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Ser Gly Leu Gly
            770                 775                 780

atc aac gct gtg gcg tat tac cgg ggg ctc gat gtg tcc gtc ata cca    3542
Ile Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val Ile Pro
        785                 790                 795

act atc gga gac gtc gtt gtc gtg gca aca gac gct ctg atg acg ggc    3590
Thr Ile Gly Asp Val Val Val Val Ala Thr Asp Ala Leu Met Thr Gly
    800                 805                 810

tat acg ggc gac ttt gac tca gtg atc gac tgt aac aca tgt gtc acc    3638
Tyr Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Thr Cys Val Thr
815                 820                 825                 830

cag aca gtc gac ttc agc ttg gat ccc acc ttc acc att gag acg acg    3686
Gln Thr Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile Glu Thr Thr
                835                 840                 845

acc gtg cct caa gac gca gtg tcg cgc tcg cag cgg cgg ggt agg act    3734
Thr Val Pro Gln Asp Ala Val Ser Arg Ser Gln Arg Arg Gly Arg Thr
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Gly Arg Gly Arg Arg Gly Ile Tyr Arg Phe Val Thr Pro Gly Glu Arg
        865                 870                 875

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Pro Ser Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys Tyr Asp Ala
    880                 885                 890

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Gly Cys Ala Trp Tyr Glu Leu Thr Pro Ala Glu Thr Ser Val Arg Leu
895                 900                 905                 910

cgg gcc tac ctg aac aca cca ggg ttg ccc gtt tgc cag gac cac ctg    3926
Arg Ala Tyr Leu Asn Thr Pro Gly Leu Pro Val Cys Gln Asp His Leu
                915                 920                 925

gag ttc tgg gag agt gtc ttc aca ggc ctc acc cat ata gat gca cac    3974
Glu Phe Trp Glu Ser Val Phe Thr Gly Leu Thr His Ile Asp Ala His
            930                 935                 940

ttc ttg tcc cag acc aag cag gca gga gac aac ttc ccc tac ctg gta    4022
Phe Leu Ser Gln Thr Lys Gln Ala Gly Asp Asn Phe Pro Tyr Leu Val
        945                 950                 955

gca tac caa gcc acg gtg tgc gcc agg gct cag gcc cca cct cca tca    4070
Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro Pro Pro Ser
    960                 965                 970

tgg gat caa atg tgg aag tgt ctc ata cgg ctg aaa cct acg ctg cac    4118
Trp Asp Gln Met Trp Lys Cys Leu Ile Arg Leu Lys Pro Thr Leu His
975                 980                 985                 990
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                995                 1000                1005

acc ctc acc cac ccc ata acc aaa tac atc atg gca tgc atg tcg gct    4214
Thr Leu Thr His Pro Ile Thr Lys Tyr Ile Met Ala Cys Met Ser Ala
            1010                1015                1020

gac ctg gag gtc gtc act agc acc tgg gtg ctg gtg ggc gga gtc ctt    4262
Asp Leu Glu Val Val Thr Ser Thr Trp Val Leu Val Gly Gly Val Leu
        1025                1030                1035

gca gct ctg gcc gcg tat tgc ctg aca aca ggc agt gtg gtc att gtg    4310
Ala Ala Leu Ala Ala Tyr Cys Leu Thr Thr Gly Ser Val Val Ile Val
    1040                1045                1050

ggt agg att atc ttg tcc ggg agg ccg gcc att gtt ccc gac agg gag    4358
Gly Arg Ile Ile Leu Ser Gly Arg Pro Ala Ile Val Pro Asp Arg Glu
1055                1060                1065                1070

ctt ctc tac cag gag ttc gat gaa atg gaa gag tgc gcc tcg cac ctc    4406
Leu Leu Tyr Gln Glu Phe Asp Glu Met Glu Glu Cys Ala Ser His Leu
                1075                1080                1085

cct tac atc gag cag gga atg cag ctc gcc gag caa ttc aag cag aaa    4454
Pro Tyr Ile Glu Gln Gly Met Gln Leu Ala Glu Gln Phe Lys Gln Lys
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gcg ctc ggg tta ctg caa aca gcc acc aaa caa gcg gag gct gct gct    4502
Ala Leu Gly Leu Leu Gln Thr Ala Thr Lys Gln Ala Glu Ala Ala Ala
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Pro Val Val Glu Ser Lys Trp Arg Ala Leu Glu Thr Phe Trp Ala Lys
    1120                1125                1130

cac atg tgg aat ttc atc agc ggg ata cag tac tta gca ggc tta tcc    4598
His Met Trp Asn Phe Ile Ser Gly Ile Gln Tyr Leu Ala Gly Leu Ser
1135                1140                1145                1150

act ctg cct ggg aac ccc gca ata gca tca ttg atg gca ttc aca gcc    4646
Thr Leu Pro Gly Asn Pro Ala Ile Ala Ser Leu Met Ala Phe Thr Ala
                1155                1160                1165

tct atc acc agc ccg ctc acc acc caa agt acc ctc ctg ttt aac atc    4694
Ser Ile Thr Ser Pro Leu Thr Thr Gln Ser Thr Leu Leu Phe Asn Ile
            1170                1175                1180

ttg ggg ggg tgg gtg gct gcc caa ctc gcc ccc ccc agc gcc gct tcg    4742
Leu Gly Gly Trp Val Ala Ala Gln Leu Ala Pro Pro Ser Ala Ala Ser
        1185                1190                1195

gct ttc gtg ggc gcc ggc atc gcc ggt gcg gct gtt ggc agc ata ggc    4790
Ala Phe Val Gly Ala Gly Ile Ala Gly Ala Ala Val Gly Ser Ile Gly
    1200                1205                1210

ctt ggg aag gtg ctt gtg gac att ctg gcg ggt tat gga gca gga gtg    4838
Leu Gly Lys Val Leu Val Asp Ile Leu Ala Gly Tyr Gly Ala Gly Val
1215                1220                1225                1230
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gcc ggc gcg ctc gtg gcc ttt aag gtc atg agc ggc gag atg ccc tcc 4886
Ala Gly Ala Leu Val Ala Phe Lys Val Met Ser Gly Glu Met Pro Ser
1235 1240 1245

acc gag gac ctg gtc aat cta ctt cct gcc atc ctc gag gaa gct agt 4934
Thr Glu Asp Leu Val Asn Leu Leu Pro Ala Ile Leu Glu Glu Ala Ser
1250 1255 1260

gag gat gtc gtc tgc tgc tca atg tcc tac aca tgg aca ggc gcc ttg 4982
Glu Asp Val Val Cys Cys Ser Met Ser Tyr Thr Trp Thr Gly Ala Leu
1265 1270 1275

gag ctg ctg ctg ctg ctg ctg ctg ggc ctg agg cta cag ctc tcc ctg 5030
Glu Leu Leu Leu Leu Leu Leu Leu Gly Leu Arg Leu Gln Leu Ser Leu
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ggc atc atc cca gtt gag gag gag aac ccg gac ttc tgg aac cgc gag 5078
Gly Ile Ile Pro Val Glu Glu Glu Asn Pro Asp Phe Trp Asn Arg Glu
1295 1300 1305 1310

gca gcc gag gcc ctg ggt gcc gcc aag aag ctg cag cct gca cag aca 5126
Ala Ala Glu Ala Leu Gly Ala Ala Lys Lys Leu Gln Pro Ala Gln Thr
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gcc gcc aag aac ctc atc atc ttc ctg ggc gat ggg atg ggg gtg tct 5174
Ala Ala Lys Asn Leu Ile Ile Phe Leu Gly Asp Gly Met Gly Val Ser
1330 1335 1340

acg gtg aca gct gcc agg atc cta aaa ggg cag aag aag gac aaa ctg 5222
Thr Val Thr Ala Ala Arg Ile Leu Lys Gly Gln Lys Lys Asp Lys Leu
1345 1350 1355

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Ala Thr Ala Tyr Leu Cys Gly Val Lys Gly Asn Phe Gln Thr Ile Gly
1395 1400 1405

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Leu Ser Ala Ala Ala Arg Phe Asn Gln Cys Asn Thr Thr Arg Gly Asn
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gag gtc atc tcc gtg atg aat cgg gcc aag aaa gca ggg aag tca gtg 5462
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Gly Val Val Thr Thr Thr Arg Val Gln His Ala Ser Pro Ala Gly Thr
1440 1445 1450

tac gcc cac acg gtg aac cgc aac tgg tac tcg gac gcc gac gtg cct 5558
Tyr Ala His Thr Val Asn Arg Asn Trp Tyr Ser Asp Ala Asp Val Pro
1455 1460 1465 1470

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            1475                1480                1485

tcc aac atg gac att gac gtg atc cta ggt gga ggc cga aag tac atg     5654
Ser Asn Met Asp Ile Asp Val Ile Leu Gly Gly Gly Arg Lys Tyr Met
        1490                1495                1500

ttt ccc atg gga acc cca gac cct gag tac cca gat gac tac agc caa     5702
Phe Pro Met Gly Thr Pro Asp Pro Glu Tyr Pro Asp Asp Tyr Ser Gln
    1505                1510                1515

ggt ggg acc agg ctg gac ggg aag aat ctg gtg cag gaa tgg ctg gcg     5750
Gly Gly Thr Arg Leu Asp Gly Lys Asn Leu Val Gln Glu Trp Leu Ala
1520                1525                1530

aag cgc cag ggt gcc cgg tat gtg tgg aac cgc act gag ctg atg cag     5798
Lys Arg Gln Gly Ala Arg Tyr Val Trp Asn Arg Thr Glu Leu Met Gln
1535                1540                1545                1550

gct tcc ctg gac ccg tct gtg acc cat ctc atg ggt ctc ttt gag cct     5846
Ala Ser Leu Asp Pro Ser Val Thr His Leu Met Gly Leu Phe Glu Pro
            1555                1560                1565

gga gac atg aaa tac gag atc cac cga gac tcc aca ctg gac ccc tcc     5894
Gly Asp Met Lys Tyr Glu Ile His Arg Asp Ser Thr Leu Asp Pro Ser
        1570                1575                1580

ctg atg gag atg aca gag gct gcc ctg cgc ctg ctg agc agg aac ccc     5942
Leu Met Glu Met Thr Glu Ala Ala Leu Arg Leu Leu Ser Arg Asn Pro
    1585                1590                1595

cgc ggc ttc ttc ctc ttc gtg gag ggt ggt cgc atc gac cat ggt cat     5990
Arg Gly Phe Phe Leu Phe Val Glu Gly Gly Arg Ile Asp His Gly His
1600                1605                1610

cat gaa agc agg gct tac cgg gca ctg act gag acg atc atg ttc gac     6038
His Glu Ser Arg Ala Tyr Arg Ala Leu Thr Glu Thr Ile Met Phe Asp
1615                1620                1625                1630

gac gcc att gag agg gcg ggc cag ctc acc agc gag gag gac acg ctg     6086
Asp Ala Ile Glu Arg Ala Gly Gln Leu Thr Ser Glu Glu Asp Thr Leu
            1635                1640                1645

agc ctc gtc act gcc gac cac tcc cac gtc ttc tcc ttc gga ggc tac     6134
Ser Leu Val Thr Ala Asp His Ser His Val Phe Ser Phe Gly Gly Tyr
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ccc ctg cga ggg agc tgc atc ttc ggg ctg gcc cct ggc aag gcc cgg     6182
Pro Leu Arg Gly Ser Cys Ile Phe Gly Leu Ala Pro Gly Lys Ala Arg
    1665                1670                1675

gac agg aag gcc tac acg gtc ctc cta tac gga aac ggt cca ggc tat     6230
Asp Arg Lys Ala Tyr Thr Val Leu Leu Tyr Gly Asn Gly Pro Gly Tyr
1680                1685                1690

gtg ctc aag gac ggc gcc cgg ccg gat gtt acc gag agc gag agc ggg     6278
Val Leu Lys Asp Gly Ala Arg Pro Asp Val Thr Glu Ser Glu Ser Gly
1695                1700                1705                1710
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Ser Pro Glu Tyr Arg Gln Gln Ser Ala Val Pro Leu Asp Glu Glu Thr
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His Ala Gly Glu Asp Val Ala Val Phe Ala Arg Gly Pro Gln Ala His
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ctg gtt cac ggc gtg cag gag cag acc ttc ata gcg cac gtc atg gcc 6422
Leu Val His Gly Val Gln Glu Gln Thr Phe Ile Ala His Val Met Ala
1745 1750 1755

ttc gcc gcc tgc ctg gag ccc tac acc gcc tgc gac ctg gcg ccc ccc 6470
Phe Ala Ala Cys Leu Glu Pro Tyr Thr Ala Cys Asp Leu Ala Pro Pro
1760 1765 1770

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Ala Gly Thr Thr Asp Ala Ala His Pro Gly
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caggctggga cacttcac atg agc gaa aaa tac atc gtc acc tgg gac atg   8611
                   Met Ser Glu Lys Tyr Ile Val Thr Trp Asp Met
                   1785                1790                1795

ttg cag atc cat gca cgt aaa ctc gca agc cga ctg atg cct tct gaa   8659
Leu Gln Ile His Ala Arg Lys Leu Ala Ser Arg Leu Met Pro Ser Glu
                1800                1805                1810

caa tgg aaa ggc att att gcc gta agc cgt ggc ggt ctg gta ccg ggt   8707
Gln Trp Lys Gly Ile Ile Ala Val Ser Arg Gly Gly Leu Val Pro Gly
            1815                1820                1825

gcg tta ctg gcg cgt gaa ctg ggt att cgt cat gtc gat acc gtt tgt   8755
Ala Leu Leu Ala Arg Glu Leu Gly Ile Arg His Val Asp Thr Val Cys
        1830                1835                1840

att tcc agc tac gat cac gac aac cag cgc gag ctt aaa gtg ctg aaa   8803
Ile Ser Ser Tyr Asp His Asp Asn Gln Arg Glu Leu Lys Val Leu Lys
    1845                1850                1855

cgc gca gaa ggc gat ggc gaa ggc ttc atc gtt att gat gac ctg gtg   8851
Arg Ala Glu Gly Asp Gly Glu Gly Phe Ile Val Ile Asp Asp Leu Val
1860                1865                1870                1875

gat acc ggt ggt act gcg gtt gcg att cgt gaa atg tat cca aaa gcg   8899
Asp Thr Gly Gly Thr Ala Val Ala Ile Arg Glu Met Tyr Pro Lys Ala
                1880                1885                1890

cac ttt gtc acc atc ttc gca aaa ccg gct ggt cgt ccg ctg gtt gat   8947
His Phe Val Thr Ile Phe Ala Lys Pro Ala Gly Arg Pro Leu Val Asp
            1895                1900                1905
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gac tat gtt gtt gat atc ccg caa gat acc tgg att gaa cag ccg tgg    8995
Asp Tyr Val Val Asp Ile Pro Gln Asp Thr Trp Ile Glu Gln Pro Trp
        1910                1915                1920

gat atg ggc gtc gta ttc gtc ccg cca atc tcc ggt cgc taatcttttc    9044
Asp Met Gly Val Val Phe Val Pro Pro Ile Ser Gly Arg
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gatcaa ttc tgt gag cgt atg gca aac gaa gga aaa ata gtt ata gta    10232
       Phe Cys Glu Arg Met Ala Asn Glu Gly Lys Ile Val Ile Val
                1940                1945                1950

gcc gca ctc gat ggg aca ttt caa cgt aaa ccg ttt aat aat att ttg    10280
Ala Ala Leu Asp Gly Thr Phe Gln Arg Lys Pro Phe Asn Asn Ile Leu
            1955                1960                1965

aat ctt att cca tta tct gaa atg gtg gta aaa cta act gct gtg tgt    10328
Asn Leu Ile Pro Leu Ser Glu Met Val Val Lys Leu Thr Ala Val Cys
        1970                1975                1980

atg aaa tgc ttt aag gag gct tcc ttt tct aaa cga ttg ggt gag gaa    10376
Met Lys Cys Phe Lys Glu Ala Ser Phe Ser Lys Arg Leu Gly Glu Glu
    1985                1990                1995
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acc gag ata gaa ata ata gga ggt aat gat atg tat caa tcg gtg tgt   10424
Thr Glu Ile Glu Ile Ile Gly Gly Asn Asp Met Tyr Gln Ser Val Cys
    2000                2005                2010

aga aag tgt tac atc gac tca taatattata ttttttatct aaaaaactaa      10475
Arg Lys Cys Tyr Ile Asp Ser
2015                2020

aaataaacat tgattaaatt ttaatataat acttaaaaat ggatgttgtg tcgttagata 10535

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                        Met Ser Ile Gln His Phe Arg Val Ala Leu
                                    2025                2030
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Ile Pro Phe Phe Ala Ala Phe Cys Leu Pro Val Phe Ala His Pro Glu
        2035                2040                2045

acg ctg gtg aaa gta aaa gat gct gaa gat cag ttg ggt gca cga gtg   12002
Thr Leu Val Lys Val Lys Asp Ala Glu Asp Gln Leu Gly Ala Arg Val
    2050                2055                2060

ggt tac atc gaa ctg gat ctc aac agc ggt aag atc ctt gag agt ttt   12050
Gly Tyr Ile Glu Leu Asp Leu Asn Ser Gly Lys Ile Leu Glu Ser Phe
2065                2070                2075

cgc ccc gaa gaa cgt ttt cca atg atg agc act ttt aaa gtt ctg cta   12098
Arg Pro Glu Glu Arg Phe Pro Met Met Ser Thr Phe Lys Val Leu Leu
2080                2085                2090                2095

tgt ggc gcg gta tta tcc cgt att gac gcc ggg caa gag caa ctc ggt   12146
Cys Gly Ala Val Leu Ser Arg Ile Asp Ala Gly Gln Glu Gln Leu Gly
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Arg Arg Ile His Tyr Ser Gln Asn Asp Leu Val Glu Tyr Ser Pro Val
            2115                2120                2125

aca gaa aag cat ctt acg gat ggc atg aca gta aga gaa tta tgc agt   12242
Thr Glu Lys His Leu Thr Asp Gly Met Thr Val Arg Glu Leu Cys Ser
        2130                2135                2140

gct gcc ata acc atg agt gat aac act gcg gcc aac tta ctt ctg aca   12290
Ala Ala Ile Thr Met Ser Asp Asn Thr Ala Ala Asn Leu Leu Leu Thr
    2145                2150                2155

acg atc gga gga ccg aag gag cta acc gct ttt ttg cac aac atg ggg   12338
Thr Ile Gly Gly Pro Lys Glu Leu Thr Ala Phe Leu His Asn Met Gly
2160                2165                2170                2175

gat cat gta act cgc ctt gat cgt tgg gaa ccg gag ctg aat gaa gcc   12386
Asp His Val Thr Arg Leu Asp Arg Trp Glu Pro Glu Leu Asn Glu Ala
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ata cca aac gac gag cgt gac acc acg atg cct gta gca atg gca aca   12434
Ile Pro Asn Asp Glu Arg Asp Thr Thr Met Pro Val Ala Met Ala Thr
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acg ttg cgc aaa cta tta act ggc gaa cta ctt act cta gct tcc cgg   12482
Thr Leu Arg Lys Leu Leu Thr Gly Glu Leu Leu Thr Leu Ala Ser Arg
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Gln Gln Leu Ile Asp Trp Met Glu Ala Asp Lys Val Ala Gly Pro Leu
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ctg cgc tcg gcc ctt ccg gct ggc tgg ttt att gct gat aaa tct gga   12578
Leu Arg Ser Ala Leu Pro Ala Gly Trp Phe Ile Ala Asp Lys Ser Gly
2240                2245                2250                2255

gcc ggt gag cgt ggg tct cgc ggt atc att gca gca ctg ggg cca gat   12626
Ala Gly Glu Arg Gly Ser Arg Gly Ile Ile Ala Ala Leu Gly Pro Asp
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act atg gat gaa cga aat aga cag atc gct gag ata ggt gcc tca ctg     12722
Thr Met Asp Glu Arg Asn Arg Gln Ile Ala Glu Ile Gly Ala Ser Leu
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Ile Lys His Trp
    2305

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Tyr Lys Cys Val Thr Ile Lys Tyr Ser Asn Asp Asn Arg Tyr Gly Thr
35 40 45

Gly Leu Trp Thr His Asp Lys Asn Asn Phe Glu Ala Leu Glu Ala Thr
50 55 60

Lys Leu Cys Asp Val Leu Glu Ser Ile Thr Asp Phe Ser Val Ile Gly
65 70 75 80

Ile Asp Glu Gly Gln Phe Phe Pro Asp Ile Val Glu Met Gly Ile Pro
85 90 95

Gln Phe Met Ala Arg Val Cys Ala Cys Leu Trp Met Met Leu Leu Ile
100 105 110

Ala Gln Ala Glu Ala Ala Leu Glu Asn Leu Val Val Leu Asn Ala Ala
115 120 125

Ser Val Ala Gly Ala His Gly Ile Leu Ser Phe Leu Val Phe Phe Cys
130 135 140

Ala Ala Trp Tyr Ile Lys Gly Arg Leu Val Pro Gly Ala Ala Tyr Ala
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Leu Tyr Gly Val Trp Pro Leu Leu Leu Leu Leu Leu Ala Leu Pro Pro
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Arg Ala Tyr Ala Met Asp Arg Glu Met Ala Ala Ser Cys Gly Gly Ala
180 185 190

Val Phe Val Gly Leu Val Leu Leu Thr Leu Ser Pro Tyr Tyr Lys Val
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Phe Leu Ala Arg Leu Ile Trp Trp Leu Gln Tyr Phe Thr Thr Arg Ala
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Glu Ala His Leu His Val Trp Ile Pro Pro Leu Asn Ala Arg Gly Gly
225 230 235 240

Arg Asp Ala Ile Ile Leu Leu Met Cys Ala Val His Pro Glu Leu Ile
245 250 255

Phe Asp Ile Thr Lys Leu Leu Ile Ala Ile Leu Gly Pro Leu Met Val
260 265 270

Leu Gln Ala Gly Ile Thr Arg Val Pro Tyr Phe Val Arg Ala Gln Gly
275 280 285

Leu Ile His Ala Cys Met Leu Val Arg Lys Val Ala Gly Gly His Tyr
290 295 300

Val Gln Met Ala Phe Met Lys Leu Gly Ala Leu Thr Gly Thr Tyr Ile
305 310 315 320

Tyr Asn His Leu Thr Pro Leu Arg Asp Trp Ala His Ala Gly Leu Arg
325 330 335

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Lys Ile Ile Thr Trp Gly Ala Asp Thr Ala Ala Cys Gly Asp Ile Ile
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Lys Gly Pro Ile Thr Gln Met Tyr Thr Asn Val Asp Gln Asp Leu Val
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Arg Ser Pro Val Phe Thr Asp Asn Ser Ser Pro Pro Ala Val Pro Gln
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Lys Ala His Gly Ile Asp Pro Asn Ile Arg Thr Gly Val Arg Thr Ile
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Cys His Ser Thr Asp Ser Thr Thr Ile Leu Gly Ile Gly Thr Val Leu
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Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Val Val Leu Ala Thr Ala
705 710 715 720

Thr Pro Pro Gly Ser Val Thr Val Pro His Pro Asn Ile Glu Glu Val
725 730 735

Ala Leu Ser Asn Thr Gly Glu Ile Pro Phe Tyr Gly Lys Ala Ile Pro
740 745 750

Ile Glu Ala Ile Arg Gly Gly Arg His Leu Ile Phe Cys His Ser Lys
755 760 765

Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Ser Gly Leu Gly Ile Asn
770 775 780

Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val Ile Pro Thr Ile
785 790 795 800

Gly Asp Val Val Val Val Ala Thr Asp Ala Leu Met Thr Gly Tyr Thr
805 810 815

Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Thr Cys Val Thr Gln Thr
820 825 830

Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile Glu Thr Thr Thr Val
835 840 845

Pro Gln Asp Ala Val Ser Arg Ser Gln Arg Arg Gly Arg Thr Gly Arg
850 855 860

Gly Arg Arg Gly Ile Tyr Arg Phe Val Thr Pro Gly Glu Arg Pro Ser
865 870 875 880

Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys Tyr Asp Ala Gly Cys
885 890 895

Ala Trp Tyr Glu Leu Thr Pro Ala Glu Thr Ser Val Arg Leu Arg Ala
900 905 910

Tyr Leu Asn Thr Pro Gly Leu Pro Val Cys Gln Asp His Leu Glu Phe
915 920 925

Trp Glu Ser Val Phe Thr Gly Leu Thr His Ile Asp Ala His Phe Leu
930 935 940

Ser Gln Thr Lys Gln Ala Gly Asp Asn Phe Pro Tyr Leu Val Ala Tyr
945 950 955 960

Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro Pro Pro Ser Trp Asp
965 970 975

Gln Met Trp Lys Cys Leu Ile Arg Leu Lys Pro Thr Leu His Gly Pro
980 985 990

Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln Asn Glu Val Thr Leu
995 1000 1005

Thr His Pro Ile Thr Lys Tyr Ile Met Ala Cys Met Ser Ala Asp Leu
1010 1015 1020

Glu Val Val Thr Ser Thr Trp Val Leu Val Gly Gly Val Leu Ala Ala
025 1030 1035 1040

Leu Ala Ala Tyr Cys Leu Thr Thr Gly Ser Val Val Ile Val Gly Arg
1045 1050 1055

Ile Ile Leu Ser Gly Arg Pro Ala Ile Val Pro Asp Arg Glu Leu Leu
1060 1065 1070

Tyr Gln Glu Phe Asp Glu Met Glu Glu Cys Ala Ser His Leu Pro Tyr
1075 1080 1085

Ile Glu Gln Gly Met Gln Leu Ala Glu Gln Phe Lys Gln Lys Ala Leu
1090 1095 1100

Gly Leu Leu Gln Thr Ala Thr Lys Gln Ala Glu Ala Ala Ala Pro Val
105 1110 1115 1120

Val Glu Ser Lys Trp Arg Ala Leu Glu Thr Phe Trp Ala Lys His Met
1125 1130 1135

Trp Asn Phe Ile Ser Gly Ile Gln Tyr Leu Ala Gly Leu Ser Thr Leu
1140 1145 1150

Pro Gly Asn Pro Ala Ile Ala Ser Leu Met Ala Phe Thr Ala Ser Ile
1155 1160 1165

Thr Ser Pro Leu Thr Thr Gln Ser Thr Leu Leu Phe Asn Ile Leu Gly
1170 1175 1180

Gly Trp Val Ala Ala Gln Leu Ala Pro Pro Ser Ala Ala Ser Ala Phe
185 1190 1195 1200

Val Gly Ala Gly Ile Ala Gly Ala Ala Val Gly Ser Ile Gly Leu Gly
1205 1210 1215

Lys Val Leu Val Asp Ile Leu Ala Gly Tyr Gly Ala Gly Val Ala Gly
1220 1225 1230

Ala Leu Val Ala Phe Lys Val Met Ser Gly Glu Met Pro Ser Thr Glu
1235 1240 1245

Asp Leu Val Asn Leu Leu Pro Ala Ile Leu Glu Glu Ala Ser Glu Asp
1250 1255 1260

Val Val Cys Cys Ser Met Ser Tyr Thr Trp Thr Gly Ala Leu Glu Leu
265 1270 1275 1280

Leu Leu Leu Leu Leu Leu Gly Leu Arg Leu Gln Leu Ser Leu Gly Ile
1285 1290 1295

Ile Pro Val Glu Glu Glu Asn Pro Asp Phe Trp Asn Arg Glu Ala Ala
1300 1305 1310

Glu Ala Leu Gly Ala Ala Lys Lys Leu Gln Pro Ala Gln Thr Ala Ala
1315 1320 1325

Lys Asn Leu Ile Ile Phe Leu Gly Asp Gly Met Gly Val Ser Thr Val
1330 1335 1340

Thr Ala Ala Arg Ile Leu Lys Gly Gln Lys Lys Asp Lys Leu Gly Pro
345 1350 1355 1360

Glu Ile Pro Leu Ala Met Asp Arg Phe Pro Tyr Val Ala Leu Ser Lys
1365 1370 1375

Thr Tyr Asn Val Asp Lys His Val Pro Asp Ser Gly Ala Thr Ala Thr
1380 1385 1390

Ala Tyr Leu Cys Gly Val Lys Gly Asn Phe Gln Thr Ile Gly Leu Ser
1395 1400 1405

Ala Ala Ala Arg Phe Asn Gln Cys Asn Thr Thr Arg Gly Asn Glu Val
1410 1415 1420

Ile Ser Val Met Asn Arg Ala Lys Lys Ala Gly Lys Ser Val Gly Val
425 1430 1435 1440

Val Thr Thr Thr Arg Val Gln His Ala Ser Pro Ala Gly Thr Tyr Ala
1445 1450 1455

His Thr Val Asn Arg Asn Trp Tyr Ser Asp Ala Asp Val Pro Ala Ser
1460 1465 1470

Ala Arg Gln Glu Gly Cys Gln Asp Ile Ala Thr Gln Leu Ile Ser Asn
1475 1480 1485

Met Asp Ile Asp Val Ile Leu Gly Gly Gly Arg Lys Tyr Met Phe Pro
1490 1495 1500

Met Gly Thr Pro Asp Pro Glu Tyr Pro Asp Asp Tyr Ser Gln Gly Gly
505 1510 1515 1520

Thr Arg Leu Asp Gly Lys Asn Leu Val Gln Glu Trp Leu Ala Lys Arg
1525 1530 1535

Gln Gly Ala Arg Tyr Val Trp Asn Arg Thr Glu Leu Met Gln Ala Ser
1540 1545 1550

Leu Asp Pro Ser Val Thr His Leu Met Gly Leu Phe Glu Pro Gly Asp
1555 1560 1565

Met Lys Tyr Glu Ile His Arg Asp Ser Thr Leu Asp Pro Ser Leu Met
1570 1575 1580

Glu Met Thr Glu Ala Ala Leu Arg Leu Leu Ser Arg Asn Pro Arg Gly
585 1590 1595 1600

Phe Phe Leu Phe Val Glu Gly Gly Arg Ile Asp His Gly His His Glu
1605 1610 1615

Ser Arg Ala Tyr Arg Ala Leu Thr Glu Thr Ile Met Phe Asp Asp Ala
1620 1625 1630

Ile Glu Arg Ala Gly Gln Leu Thr Ser Glu Glu Asp Thr Leu Ser Leu
1635 1640 1645

Val Thr Ala Asp His Ser His Val Phe Ser Phe Gly Gly Tyr Pro Leu
1650 1655 1660

Arg Gly Ser Cys Ile Phe Gly Leu Ala Pro Gly Lys Ala Arg Asp Arg
665 1670 1675 1680

Lys Ala Tyr Thr Val Leu Leu Tyr Gly Asn Gly Pro Gly Tyr Val Leu
1685 1690 1695

Lys Asp Gly Ala Arg Pro Asp Val Thr Glu Ser Glu Ser Gly Ser Pro
1700 1705 1710

Glu Tyr Arg Gln Gln Ser Ala Val Pro Leu Asp Glu Glu Thr His Ala
1715 1720 1725

Gly Glu Asp Val Ala Val Phe Ala Arg Gly Pro Gln Ala His Leu Val
1730 1735 1740

His Gly Val Gln Glu Gln Thr Phe Ile Ala His Val Met Ala Phe Ala
745 1750 1755 1760

Ala Cys Leu Glu Pro Tyr Thr Ala Cys Asp Leu Ala Pro Pro Ala Gly
1765 1770 1775

Thr Thr Asp Ala Ala His Pro Gly Met Ser Glu Lys Tyr Ile Val Thr
1780 785 1790

Trp Asp Met Leu Gln Ile His Ala Arg Lys Leu Ala Ser Arg Leu Met
1795 1800 1805

Pro Ser Glu Gln Trp Lys Gly Ile Ile Ala Val Ser Arg Gly Gly Leu
1810 1815 1820

Val Pro Gly Ala Leu Leu Ala Arg Glu Leu Gly Ile Arg His Val Asp
825 1830 1835 1840

Thr Val Cys Ile Ser Ser Tyr Asp His Asp Asn Gln Arg Glu Leu Lys
1845 1850 1855

Val Leu Lys Arg Ala Glu Gly Asp Gly Glu Gly Phe Ile Val Ile Asp
1860 1865 1870

Asp Leu Val Asp Thr Gly Gly Thr Ala Val Ala Ile Arg Glu Met Tyr
1875 1880 1885

Pro Lys Ala His Phe Val Thr Ile Phe Ala Lys Pro Ala Gly Arg Pro
1890 1895 1900

Leu Val Asp Asp Tyr Val Val Asp Ile Pro Gln Asp Thr Trp Ile Glu
905 1910 1915 1920

Gln Pro Trp Asp Met Gly Val Val Phe Val Pro Pro Ile Ser Gly Arg
1925 1930 1935

Phe Cys Glu Arg Met Ala Asn Glu Gly Lys Ile Val Ile Val Ala Ala
1940 1945 1950

Leu Asp Gly Thr Phe Gln Arg Lys Pro Phe Asn Asn Ile Leu Asn Leu
1955 1960 1965

Ile Pro Leu Ser Glu Met Val Val Lys Leu Thr Ala Val Cys Met Lys
1970 1975 1980

Cys Phe Lys Glu Ala Ser Phe Ser Lys Arg Leu Gly Glu Glu Thr Glu
985 1990 1995 2000

Ile Glu Ile Ile Gly Gly Asn Asp Met Tyr Gln Ser Val Cys Arg Lys
2005 2010 2015

Cys Tyr Ile Asp Ser Met Ser Ile Gln His Phe Arg Val Ala Leu Ile
2020 2025 2030

Pro Phe Phe Ala Ala Phe Cys Leu Pro Val Phe Ala His Pro Glu Thr
2035 2040 2045

Leu Val Lys Val Lys Asp Ala Glu Asp Gln Leu Gly Ala Arg Val Gly
2050 2055 2060

Tyr Ile Glu Leu Asp Leu Asn Ser Gly Lys Ile Leu Glu Ser Phe Arg
065 2070 2075 208

Pro Glu Glu Arg Phe Pro Met Met Ser Thr Phe Lys Val Leu Leu Cys
2085 2090 2095

Gly Ala Val Leu Ser Arg Ile Asp Ala Gly Gln Glu Gln Leu Gly Arg
2100 2105 2110

Arg Ile His Tyr Ser Gln Asn Asp Leu Val Glu Tyr Ser Pro Val Thr
2115 2120 2125

Glu Lys His Leu Thr Asp Gly Met Thr Val Arg Glu Leu Cys Ser Ala
2130 2135 2140

Ala Ile Thr Met Ser Asp Asn Thr Ala Ala Asn Leu Leu Leu Thr Thr
145 2150 2155 216

Ile Gly Gly Pro Lys Glu Leu Thr Ala Phe Leu His Asn Met Gly Asp
2165 2170 2175

His Val Thr Arg Leu Asp Arg Trp Glu Pro Glu Leu Asn Glu Ala Ile
2180 2185 2190

Pro Asn Asp Glu Arg Asp Thr Thr Met Pro Val Ala Met Ala Thr Thr
2195 2200 2205

Leu Arg Lys Leu Leu Thr Gly Glu Leu Leu Thr Leu Ala Ser Arg Gln
2210 2215 2220

Gln Leu Ile Asp Trp Met Glu Ala Asp Lys Val Ala Gly Pro Leu Leu
225 2230 2235 224

Arg Ser Ala Leu Pro Ala Gly Trp Phe Ile Ala Asp Lys Ser Gly Ala
2245 2250 2255

Gly Glu Arg Gly Ser Arg Gly Ile Ile Ala Ala Leu Gly Pro Asp Gly
2260 2265 2270

Lys Pro Ser Arg Ile Val Val Ile Tyr Thr Thr Gly Ser Gln Ala Thr
2275 2280 2285

Met Asp Glu Arg Asn Arg Gln Ile Ala Glu Ile Gly Ala Ser Leu Ile
2290 2295 2300

Lys His Trp
305

<210> 3
<211> 92
<212> PRT
<213> Artificial Sequence

<400> 3
Met Asn Gly Gly His Ile Gln Leu Ile Ile Gly Pro Met Phe Ser Gly
1 5 10 15

Lys Ser Thr Glu Leu Ile Arg Arg Val Arg Arg Tyr Gln Ile Ala Gln
20 25 30

Tyr Lys Cys Val Thr Ile Lys Tyr Ser Asn Asp Asn Arg Tyr Gly Thr
35 40 45

Gly Leu Trp Thr His Asp Lys Asn Asn Phe Glu Ala Leu Glu Ala Thr
50 55 60

Lys Leu Cys Asp Val Leu Glu Ser Ile Thr Asp Phe Ser Val Ile Gly
65 70 75 80

Ile Asp Glu Gly Gln Phe Phe Pro Asp Ile Val Glu
85 90

<210> 4
<211> 1692
<212> PRT
<213> Artificial Sequence

<400> 4
Met Gly Ile Pro Gln Phe Met Ala Arg Val Cys Ala Cys Leu Trp Met
1 5 10 15

Met Leu Leu Ile Ala Gln Ala Glu Ala Ala Leu Glu Asn Leu Val Val
20 25 30

Leu Asn Ala Ala Ser Val Ala Gly Ala His Gly Ile Leu Ser Phe Leu
35 40 45

Val Phe Phe Cys Ala Ala Trp Tyr Ile Lys Gly Arg Leu Val Pro Gly
50 55 60

Ala Ala Tyr Ala Leu Tyr Gly Val Trp Pro Leu Leu Leu Leu Leu Leu
65 70 75 80

Ala Leu Pro Pro Arg Ala Tyr Ala Met Asp Arg Glu Met Ala Ala Ser
85 90 95

Cys Gly Gly Ala Val Phe Val Gly Leu Val Leu Leu Thr Leu Ser Pro
100 105 110

Tyr Tyr Lys Val Phe Leu Ala Arg Leu Ile Trp Trp Leu Gln Tyr Phe
115 120 125

Thr Thr Arg Ala Glu Ala His Leu His Val Trp Ile Pro Pro Leu Asn
130 135 140

Ala Arg Gly Gly Arg Asp Ala Ile Ile Leu Leu Met Cys Ala Val His
145 150 155 160

Pro Glu Leu Ile Phe Asp Ile Thr Lys Leu Leu Ile Ala Ile Leu Gly
165 170 175

Pro Leu Met Val Leu Gln Ala Gly Ile Thr Arg Val Pro Tyr Phe Val
180 185 190

Arg Ala Gln Gly Leu Ile His Ala Cys Met Leu Val Arg Lys Val Ala
195 200 205

Gly Gly His Tyr Val Gln Met Ala Phe Met Lys Leu Gly Ala Leu Thr
210 215 220

Gly Thr Tyr Ile Tyr Asn His Leu Thr Pro Leu Arg Asp Trp Ala His
225 230 235 240

Ala Gly Leu Arg Asp Leu Ala Val Ala Val Glu Pro Val Val Phe Ser
245 250 255

Asp Met Glu Thr Lys Ile Ile Thr Trp Gly Ala Asp Thr Ala Ala Cys
260 265 270

Gly Asp Ile Ile Leu Gly Leu Pro Val Ser Ala Arg Arg Gly Lys Glu
275 280 285

Ile Leu Leu Gly Pro Ala Asp Ser Leu Glu Gly Arg Gly Trp Arg Leu
290 295 300

Leu Ala Pro Ile Thr Ala Tyr Ser Gln Gln Thr Arg Gly Leu Leu Gly
305 310 315 320

Cys Ile Ile Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu Gly
325 330 335

Glu Val Gln Val Val Ser Thr Ala Thr Gln Ser Phe Leu Ala Thr Cys
340 345 350

Val Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly Ser Lys Thr
355 360 365

Leu Ala Gly Pro Lys Gly Pro Ile Thr Gln Met Tyr Thr Asn Val Asp
370 375 380

Gln Asp Leu Val Gly Trp Gln Ala Pro Pro Gly Ala Arg Ser Leu Thr
385 390 395 400

Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr Leu Val Thr Arg His Ala
405 410 415

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Asp Val Ile Pro Val Arg Arg Arg Gly Asp Ser Arg Gly Ser Leu Leu
            420                 425                 430

Ser Pro Arg Pro Val Ser Tyr Leu Lys Gly Ser Ser Gly Gly Pro Leu
        435                 440                 445

Leu Cys Pro Ser Gly His Ala Val Gly Ile Phe Arg Ala Ala Val Cys
    450                 455                 460

Thr Arg Gly Val Ala Lys Ala Val Asp Phe Val Pro Val Glu Ser Met
465                 470                 475                 480

Glu Thr Thr Met Arg Ser Pro Val Phe Thr Asp Asn Ser Ser Pro Pro
                485                 490                 495

Ala Val Pro Gln Ser Phe Gln Val Ala His Leu His Ala Pro Thr Gly
            500                 505                 510

Ser Gly Lys Ser Thr Lys Val Pro Ala Ala Tyr Ala Ala Gln Gly Tyr
        515                 520                 525

Lys Val Leu Val Leu Asn Pro Ser Val Ala Ala Thr Leu Gly Phe Gly
    530                 535                 540

Ala Tyr Met Ser Lys Ala His Gly Ile Asp Pro Asn Ile Arg Thr Gly
545                 550                 555                 560

Val Arg Thr Ile Thr Thr Gly Ala Pro Val Thr Tyr Ser Thr Tyr Gly
                565                 570                 575

Lys Phe Leu Ala Asp Gly Gly Cys Ser Gly Gly Ala Tyr Asp Ile Ile
            580                 585                 590

Ile Cys Asp Glu Cys His Ser Thr Asp Ser Thr Thr Ile Leu Gly Ile
        595                 600                 605

Gly Thr Val Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Val Val
    610                 615                 620

Leu Ala Thr Ala Thr Pro Pro Gly Ser Val Thr Val Pro His Pro Asn
625                 630                 635                 640

Ile Glu Glu Val Ala Leu Ser Asn Thr Gly Glu Ile Pro Phe Tyr Gly
                645                 650                 655

Lys Ala Ile Pro Ile Glu Ala Ile Arg Gly Gly Arg His Leu Ile Phe
            660                 665                 670

Cys His Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Ser Gly
        675                 680                 685

Leu Gly Ile Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val
    690                 695                 700

Ile Pro Thr Ile Gly Asp Val Val Val Val Ala Thr Asp Ala Leu Met
705                 710                 715                 720

Thr Gly Tyr Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Thr Cys
                725                 730                 735
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Val Thr Gln Thr Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile Glu
740 745 750

Thr Thr Thr Val Pro Gln Asp Ala Val Ser Arg Ser Gln Arg Arg Gly
755 760 765

Arg Thr Gly Arg Gly Arg Arg Gly Ile Tyr Arg Phe Val Thr Pro Gly
770 775 780

Glu Arg Pro Ser Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys Tyr
785 790 795 800

Asp Ala Gly Cys Ala Trp Tyr Glu Leu Thr Pro Ala Glu Thr Ser Val
805 810 815

Arg Leu Arg Ala Tyr Leu Asn Thr Pro Gly Leu Pro Val Cys Gln Asp
820 825 830

His Leu Glu Phe Trp Glu Ser Val Phe Thr Gly Leu Thr His Ile Asp
835 840 845

Ala His Phe Leu Ser Gln Thr Lys Gln Ala Gly Asp Asn Phe Pro Tyr
850 855 860

Leu Val Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro Pro
865 870 875 880

Pro Ser Trp Asp Gln Met Trp Lys Cys Leu Ile Arg Leu Lys Pro Thr
885 890 895

Leu His Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln Asn
900 905 910

Glu Val Thr Leu Thr His Pro Ile Thr Lys Tyr Ile Met Ala Cys Met
915 920 925

Ser Ala Asp Leu Glu Val Val Thr Ser Thr Trp Val Leu Val Gly Gly
930 935 940

Val Leu Ala Ala Leu Ala Ala Tyr Cys Leu Thr Thr Gly Ser Val Val
945 950 955 960

Ile Val Gly Arg Ile Ile Leu Ser Gly Arg Pro Ala Ile Val Pro Asp
965 970 975

Arg Glu Leu Leu Tyr Gln Glu Phe Asp Glu Met Glu Glu Cys Ala Ser
980 985 990

His Leu Pro Tyr Ile Glu Gln Gly Met Gln Leu Ala Glu Gln Phe Lys
995 1000 1005

Gln Lys Ala Leu Gly Leu Leu Gln Thr Ala Thr Lys Gln Ala Glu Ala
1010 1015 1020

Ala Ala Pro Val Val Glu Ser Lys Trp Arg Ala Leu Glu Thr Phe Trp
025 1030 1035 1040

Ala Lys His Met Trp Asn Phe Ile Ser Gly Ile Gln Tyr Leu Ala Gly
1045 1050 1055

Leu Ser Thr Leu Pro Gly Asn Pro Ala Ile Ala Ser Leu Met Ala Phe
1060 1065 1070

Thr Ala Ser Ile Thr Ser Pro Leu Thr Thr Gln Ser Thr Leu Leu Phe
1075 1080 1085

Asn Ile Leu Gly Gly Trp Val Ala Ala Gln Leu Ala Pro Pro Ser Ala
1090 1095 1100

Ala Ser Ala Phe Val Gly Ala Gly Ile Ala Gly Ala Ala Val Gly Ser
105 1110 1115 1120

Ile Gly Leu Gly Lys Val Leu Val Asp Ile Leu Ala Gly Tyr Gly Ala
1125 1130 1135

Gly Val Ala Gly Ala Leu Val Ala Phe Lys Val Met Ser Gly Glu Met
1140 1145 1150

Pro Ser Thr Glu Asp Leu Val Asn Leu Leu Pro Ala Ile Leu Glu Glu
1155 1160 1165

Ala Ser Glu Asp Val Val Cys Cys Ser Met Ser Tyr Thr Trp Thr Gly
1170 1175 1180

Ala Leu Glu Leu Leu Leu Leu Leu Leu Leu Gly Leu Arg Leu Gln Leu
185 1190 1195 1200

Ser Leu Gly Ile Ile Pro Val Glu Glu Glu Asn Pro Asp Phe Trp Asn
1205 1210 1215

Arg Glu Ala Ala Glu Ala Leu Gly Ala Ala Lys Lys Leu Gln Pro Ala
1220 1225 1230

Gln Thr Ala Ala Lys Asn Leu Ile Ile Phe Leu Gly Asp Gly Met Gly
1235 1240 1245

Val Ser Thr Val Thr Ala Ala Arg Ile Leu Lys Gly Gln Lys Lys Asp
1250 1255 1260

Lys Leu Gly Pro Glu Ile Pro Leu Ala Met Asp Arg Phe Pro Tyr Val
265 1270 1275 1280

Ala Leu Ser Lys Thr Tyr Asn Val Asp Lys His Val Pro Asp Ser Gly
1285 1290 1295

Ala Thr Ala Thr Ala Tyr Leu Cys Gly Val Lys Gly Asn Phe Gln Thr
1300 1305 1310

Ile Gly Leu Ser Ala Ala Ala Arg Phe Asn Gln Cys Asn Thr Thr Arg
1315 1320 1325

Gly Asn Glu Val Ile Ser Val Met Asn Arg Ala Lys Lys Ala Gly Lys
1330 1335 1340

Ser Val Gly Val Val Thr Thr Thr Arg Val Gln His Ala Ser Pro Ala
345 1350 1355 1360

Gly Thr Tyr Ala His Thr Val Asn Arg Asn Trp Tyr Ser Asp Ala Asp
1365 1370 1375

Val Pro Ala Ser Ala Arg Gln Glu Gly Cys Gln Asp Ile Ala Thr Gln
1380 1385 1390

Leu Ile Ser Asn Met Asp Ile Asp Val Ile Leu Gly Gly Gly Arg Lys
1395 1400 1405

Tyr Met Phe Pro Met Gly Thr Pro Asp Pro Glu Tyr Pro Asp Asp Tyr
1410 1415 1420

Ser Gln Gly Gly Thr Arg Leu Asp Gly Lys Asn Leu Val Gln Glu Trp
425 1430 1435 1440

Leu Ala Lys Arg Gln Gly Ala Arg Tyr Val Trp Asn Arg Thr Glu Leu
1445 1450 1455

Met Gln Ala Ser Leu Asp Pro Ser Val Thr His Leu Met Gly Leu Phe
1460 1465 1470

Glu Pro Gly Asp Met Lys Tyr Glu Ile His Arg Asp Ser Thr Leu Asp
1475 1480 1485

Pro Ser Leu Met Glu Met Thr Glu Ala Ala Leu Arg Leu Leu Ser Arg
1490 1495 1500

Asn Pro Arg Gly Phe Phe Leu Phe Val Glu Gly Gly Arg Ile Asp His
505 1510 1515 1520

Gly His His Glu Ser Arg Ala Tyr Arg Ala Leu Thr Glu Thr Ile Met
1525 1530 1535

Phe Asp Asp Ala Ile Glu Arg Ala Gly Gln Leu Thr Ser Glu Glu Asp
1540 1545 1550

Thr Leu Ser Leu Val Thr Ala Asp His Ser His Val Phe Ser Phe Gly
1555 1560 1565

Gly Tyr Pro Leu Arg Gly Ser Cys Ile Phe Gly Leu Ala Pro Gly Lys
1570 1575 1580

Ala Arg Asp Arg Lys Ala Tyr Thr Val Leu Leu Tyr Gly Asn Gly Pro
585 1590 1595 1600

Gly Tyr Val Leu Lys Asp Gly Ala Arg Pro Asp Val Thr Glu Ser Glu
1605 1610 1615

Ser Gly Ser Pro Glu Tyr Arg Gln Gln Ser Ala Val Pro Leu Asp Glu
1620 1625 1630

Glu Thr His Ala Gly Glu Asp Val Ala Val Phe Ala Arg Gly Pro Gln
1635 1640 1645

Ala His Leu Val His Gly Val Gln Glu Gln Thr Phe Ile Ala His Val
1650 1655 1660

Met Ala Phe Ala Ala Cys Leu Glu Pro Tyr Thr Ala Cys Asp Leu Ala
665 1670 1675 1680

Pro Pro Ala Gly Thr Thr Asp Ala Ala His Pro Gly
1685 1690

<210> 5
<211> 152
<212> PRT
<213> Artificial Sequence

<400> 5
Met Ser Glu Lys Tyr Ile Val Thr Trp Asp Met Leu Gln Ile His Ala
1 5 10 15

Arg Lys Leu Ala Ser Arg Leu Met Pro Ser Glu Gln Trp Lys Gly Ile
20 25 30

Ile Ala Val Ser Arg Gly Gly Leu Val Pro Gly Ala Leu Leu Ala Arg
35 40 45

Glu Leu Gly Ile Arg His Val Asp Thr Val Cys Ile Ser Ser Tyr Asp
50 55 60

His Asp Asn Gln Arg Glu Leu Lys Val Leu Lys Arg Ala Glu Gly Asp
65 70 75 80

Gly Glu Gly Phe Ile Val Ile Asp Asp Leu Val Asp Thr Gly Gly Thr
85 90 95

Ala Val Ala Ile Arg Glu Met Tyr Pro Lys Ala His Phe Val Thr Ile
100 105 110

Phe Ala Lys Pro Ala Gly Arg Pro Leu Val Asp Asp Tyr Val Val Asp
115 120 125

Ile Pro Gln Asp Thr Trp Ile Glu Gln Pro Trp Asp Met Gly Val Val
130 135 140

Phe Val Pro Pro Ile Ser Gly Arg
145 150

<210> 6
<211> 85
<212> PRT
<213> Artificial Sequence

<400> 6
Phe Cys Glu Arg Met Ala Asn Glu Gly Lys Ile Val Ile Val Ala Ala
1 5 10 15

Leu Asp Gly Thr Phe Gln Arg Lys Pro Phe Asn Asn Ile Leu Asn Leu
20 25 30

Ile Pro Leu Ser Glu Met Val Val Lys Leu Thr Ala Val Cys Met Lys
35 40 45

Cys Phe Lys Glu Ala Ser Phe Ser Lys Arg Leu Gly Glu Glu Thr Glu
50 55 60

Ile Glu Ile Ile Gly Gly Asn Asp Met Tyr Gln Ser Val Cys Arg Lys
65 70 75 80

Cys Tyr Ile Asp Ser
85

<210> 7
<211> 286
<212> PRT
<213> Artificial Sequence

<400> 7
Met Ser Ile Gln His Phe Arg Val Ala Leu Ile Pro Phe Phe Ala Ala
1 5 10 15

Phe Cys Leu Pro Val Phe Ala His Pro Glu Thr Leu Val Lys Val Lys
20 25 30

Asp Ala Glu Asp Gln Leu Gly Ala Arg Val Gly Tyr Ile Glu Leu Asp
35 40 45

Leu Asn Ser Gly Lys Ile Leu Glu Ser Phe Arg Pro Glu Glu Arg Phe
50 55 60

Pro Met Met Ser Thr Phe Lys Val Leu Leu Cys Gly Ala Val Leu Ser
65 70 75 80

Arg Ile Asp Ala Gly Gln Glu Gln Leu Gly Arg Arg Ile His Tyr Ser
85 90 95

Gln Asn Asp Leu Val Glu Tyr Ser Pro Val Thr Glu Lys His Leu Thr
100 105 110

Asp Gly Met Thr Val Arg Glu Leu Cys Ser Ala Ala Ile Thr Met Ser
115 120 125

Asp Asn Thr Ala Ala Asn Leu Leu Leu Thr Thr Ile Gly Gly Pro Lys
130 135 140

Glu Leu Thr Ala Phe Leu His Asn Met Gly Asp His Val Thr Arg Leu
145 150 155 160

Asp Arg Trp Glu Pro Glu Leu Asn Glu Ala Ile Pro Asn Asp Glu Arg
165 170 175

Asp Thr Thr Met Pro Val Ala Met Ala Thr Thr Leu Arg Lys Leu Leu
180 185 190

Thr Gly Glu Leu Leu Thr Leu Ala Ser Arg Gln Gln Leu Ile Asp Trp
195 200 205

Met Glu Ala Asp Lys Val Ala Gly Pro Leu Leu Arg Ser Ala Leu Pro
210 215 220

Ala Gly Trp Phe Ile Ala Asp Lys Ser Gly Ala Gly Glu Arg Gly Ser
225 230 235 240

Arg Gly Ile Ile Ala Ala Leu Gly Pro Asp Gly Lys Pro Ser Arg Ile
245 250 255

Val Val Ile Tyr Thr Thr Gly Ser Gln Ala Thr Met Asp Glu Arg Asn
260 265 270

Arg Gln Ile Ala Glu Ile Gly Ala Ser Leu Ile Lys His Trp
275 280 285

<210> 8
<211> 13910
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: plasmid phcap 3

<220>
<221> CDS
<222> (497)..(772)

<220>
<221> CDS
<222> (1425)..(6500)

<220>
<221> CDS
<222> (8579)..(9034)

<220>
<221> CDS
<222> (10191)..(10445)

<220>
<221> CDS
<222> (11877)..(12734)

<220>
<221> misc_feature
<222> (1)..(774)
<223> Vaccinia Virus thymidine Kinase gene recombination site

<220>
<221> promoter
<222> (794)..(816)
<223> T7 promoter

<220>
<221> misc_feature
<222> (846)..(1424)
<223> EMC/Internal Ribosome Entry Site (IRES)

<220>
<221> misc_feature
<222> (1426)..(1437)
<223> MCS (Multiple Cloning Site)

<220>
<221> misc_feature
<222> (1446)..(2318)
<223> HCV E2/ NS2 domain

<220>
<221> misc_feature
<222> (2319)..(4231)
<223> HCV NS3 Domain containing the serine protease and helicase enzymes

<220>
<221> misc_feature
<222> (4203)..(4260)
<223> HCV NS3-NS4A cleavage site

<220>
<221> misc_feature
<222> (4375)..(4424)
<223> HCV NS4A-4B clevage site

<220>
<221> misc_feature
<222> (4233)..(4394)
<223> HCV NS4A domain

<220>
<221> misc_feature
<222> (4395)..(4919)
<223> HCV NS4B Domain

<220>
<221> misc_feature
<222> (4920)..(4991)
<223> HCV NS5A-NS5B cleavage site

<220>
<221> misc_feature
<222> (4992)..(6501)
<223> SEAP Protein

<220>
<221> misc_feature
<222> (7915)..(7945)
<223> MCS (Multiple Cloning Site)

<220>
<221> terminator
<222> (7938)..(8078)
<223> term T7

<220>
<221> promoter
<222> (8080)..(8365)
<223> Vacinina virus promoter; early/late promoter

<220>
<221> misc_feature
<222> (8560)..(11317)
<223> E. coli gpt; for selection of recombinants

<220>
<221> misc_feature
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<223> remaining DNA from 3' end of Tropix pCMV/SEAP plasmid

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attcacagac tttcaagatt ttaaaaaact gtttaacaag gtccctattg ttacagatgg 300

aagggtcaaa cttaataaag gatatttgtt cgactttgtg attagtttga tgcgattcaa 360

aaaagaatcc tctctagcta ccaccgcaat agatcctgtt agatacatag atcctcgtcg 420

caatatcgca ttttctaacg tgatggatat attaaagtcg aataaagtga acaataatta 480

attctttatt gtcatc atg aac ggc gga cat att cag ttg ata atc ggc ccc 532
                  Met Asn Gly Gly His Ile Gln Leu Ile Ile Gly Pro
                   1               5                  10

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Met Phe Ser Gly Lys Ser Thr Glu Leu Ile Arg Arg Val Arg Arg Tyr
        15                  20                  25

caa ata gct caa tat aaa tgc gtg act ata aaa tat tct aac gat aat   628
Gln Ile Ala Gln Tyr Lys Cys Val Thr Ile Lys Tyr Ser Asn Asp Asn
    30                  35                  40

aga tac gga acg gga cta tgg acg cat gat aag aat aat ttt gaa gca   676
Arg Tyr Gly Thr Gly Leu Trp Thr His Asp Lys Asn Asn Phe Glu Ala
45                  50                  55                  60

ttg gaa gca act aaa cta tgt gat gtc ttg gaa tca att aca gat ttc   724
Leu Glu Ala Thr Lys Leu Cys Asp Val Leu Glu Ser Ile Thr Asp Phe
                65                  70                  75

tcc gtg ata ggt atc gat gaa gga cag ttc ttt cca gac att gtt gaa   772
Ser Val Ile Gly Ile Asp Glu Gly Gln Phe Phe Pro Asp Ile Val Glu
            80                  85                  90

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Leu Ile Ala Gln Ala Glu Ala Ala Leu Glu Asn Leu Val Val Leu Asn
115 120 125

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Ala Ala Ser Val Ala Gly Ala His Gly Ile Leu Ser Phe Leu Val Phe
130 135 140

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Phe Cys Ala Ala Trp Tyr Ile Lys Gly Arg Leu Val Pro Gly Ala Ala
145 150 155

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Tyr Ala Leu Tyr Gly Val Trp Pro Leu Leu Leu Leu Leu Leu Ala Leu
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Lys Val Phe Leu Ala Arg Leu Ile Trp Trp Leu Gln Tyr Phe Thr Thr
210 215 220

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Arg Ala Glu Ala His Leu His Val Trp Ile Pro Pro Leu Asn Ala Arg
225 230 235

gga ggc cgc gat gcc atc atc ctc ctc atg tgc gca gtc cat cca gag 1910
Gly Gly Arg Asp Ala Ile Ile Leu Leu Met Cys Ala Val His Pro Glu
240 245 250

cta atc ttt gac atc acc aaa ctt cta att gcc ata ctc ggt ccg ctc 1958
Leu Ile Phe Asp Ile Thr Lys Leu Leu Ile Ala Ile Leu Gly Pro Leu
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Met Val Leu Gln Ala Gly Ile Thr Arg Val Pro Tyr Phe Val Arg Ala
275 280 285

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Gln Gly Leu Ile His Ala Cys Met Leu Val Arg Lys Val Ala Gly Gly
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cat tat gtc caa atg gcc ttc atg aag ctg ggc gcg ctg aca ggc acg 2102
His Tyr Val Gln Met Ala Phe Met Lys Leu Gly Ala Leu Thr Gly Thr
305 310 315

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Tyr Ile Tyr Asn His Leu Thr Pro Leu Arg Asp Trp Ala His Ala Gly
320 325 330

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Leu Arg Asp Leu Ala Val Ala Val Glu Pro Val Val Phe Ser Asp Met
335 340 345 350

gag acc aag atc atc acc tgg gga gca gac acc gcg gcg tgt ggg gac 2246
Glu Thr Lys Ile Ile Thr Trp Gly Ala Asp Thr Ala Ala Cys Gly Asp
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Ile Ile Leu Gly Leu Pro Val Ser Ala Arg Arg Gly Lys Glu Ile Leu
370 375 380

ctg ggc ccg gcc gat agt ctt gaa ggg cgg ggg tgg cga ctc ctc gcg 2342
Leu Gly Pro Ala Asp Ser Leu Glu Gly Arg Gly Trp Arg Leu Leu Ala
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Pro Ile Thr Ala Tyr Ser Gln Gln Thr Arg Gly Leu Leu Gly Cys Ile
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Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly Ser Lys Thr Leu Ala
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Leu Val Gly Trp Gln Ala Pro Pro Gly Ala Arg Ser Leu Thr Pro Cys
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Arg Pro Val Ser Tyr Leu Lys Gly Ser Ala Gly Gly Pro Leu Leu Cys
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Pro Ser Gly His Ala Val Gly Ile Phe Arg Ala Ala Val Cys Thr Arg
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Gly Val Ala Lys Ala Val Asp Phe Val Pro Val Glu Ser Met Glu Thr
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Thr Met Arg Ser Pro Val Phe Thr Asp Asn Ser Ser Pro Pro Ala Val
575                 580                 585                 590

ccg cag tca ttt caa gtg gcc cac cta cac gct ccc act ggc agc ggc    2966
Pro Gln Ser Phe Gln Val Ala His Leu His Ala Pro Thr Gly Ser Gly
                595                 600                 605

aag agt act aaa gtg ccg gct gca tat gca gcc caa ggg tac aag gtg    3014
Lys Ser Thr Lys Val Pro Ala Ala Tyr Ala Ala Gln Gly Tyr Lys Val
            610                 615                 620

ctc gtc ctc aat ccg tcc gtt gcc gct acc tta ggg ttt ggg gcg tat    3062
Leu Val Leu Asn Pro Ser Val Ala Ala Thr Leu Gly Phe Gly Ala Tyr
    625                 630                 635

atg tct aag gca cac ggt att gac ccc aac atc aga act ggg gta agg    3110
Met Ser Lys Ala His Gly Ile Asp Pro Asn Ile Arg Thr Gly Val Arg
    640                 645                 650

acc att acc aca ggc gcc ccc gtc aca tac tct acc tat ggc aag ttt    3158
Thr Ile Thr Thr Gly Ala Pro Val Thr Tyr Ser Thr Tyr Gly Lys Phe
655                 660                 665                 670

ctt gcc gat ggt ggt tgc tct ggg ggc gct tat gac atc ata ata tgt    3206
Leu Ala Asp Gly Gly Cys Ser Gly Gly Ala Tyr Asp Ile Ile Ile Cys
                675                 680                 685

gat gag tgc cat tca act gac tcg act aca atc ttg ggc atc ggc aca    3254
Asp Glu Cys His Ser Thr Asp Ser Thr Thr Ile Leu Gly Ile Gly Thr
            690                 695                 700

gtc ctg gac caa gcg gag acg gct gga gcg cgg ctt gtc gtg ctc gcc    3302
Val Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Val Val Leu Ala
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Thr Ala Thr Pro Pro Gly Ser Val Thr Val Pro His Pro Asn Ile Glu
    720                 725                 730

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735                 740                 745                 750

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Ile Pro Ile Glu Ala Ile Arg Gly Gly Arg His Leu Ile Phe Cys His
                755                 760                 765

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Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Ser Gly Leu Gly
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atc aac gct gtg gcg tat tac cgg ggg ctc gat gtg tcc gtc ata cca    3542
Ile Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val Ile Pro
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act atc gga gac gtc gtt gtc gtg gca aca gac gct ctg atg acg ggc    3590
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Tyr Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Thr Cys Val Thr
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cag aca gtc gac ttc agc ttg gat ccc acc ttc acc att gag acg acg     3686
Gln Thr Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile Glu Thr Thr
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acc gtg cct caa gac gca gtg tcg cgc tcg cag cgg cgg ggt agg act     3734
Thr Val Pro Gln Asp Ala Val Ser Arg Ser Gln Arg Arg Gly Arg Thr
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Gly Arg Gly Arg Arg Gly Ile Tyr Arg Phe Val Thr Pro Gly Glu Arg
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Pro Ser Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys Tyr Asp Ala
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Gly Cys Ala Trp Tyr Glu Leu Thr Pro Ala Glu Thr Ser Val Arg Leu
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cgg gcc tac ctg aac aca cca ggg ttg ccc gtt tgc cag gac cac ctg     3926
Arg Ala Tyr Leu Asn Thr Pro Gly Leu Pro Val Cys Gln Asp His Leu
                915                 920                 925

gag ttc tgg gag agt gtc ttc aca ggc ctc acc cat ata gat gca cac     3974
Glu Phe Trp Glu Ser Val Phe Thr Gly Leu Thr His Ile Asp Ala His
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ttc ttg tcc cag acc aag cag gca gga gac aac ttc ccc tac ctg gta     4022
Phe Leu Ser Gln Thr Lys Gln Ala Gly Asp Asn Phe Pro Tyr Leu Val
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gca tac caa gcc acg gtg tgc gcc agg gct cag gcc cca cct cca tca     4070
Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro Pro Pro Ser
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Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln Asn Glu Val
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Thr Leu Thr His Pro Ile Thr Lys Tyr Ile Met Ala Cys Met Ser Ala
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Ala Ala Leu Ala Ala Tyr Cys Leu Thr Thr Gly Ser Val Val Ile Val
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1075 1080 1085

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Pro Tyr Ile Glu Gln Gly Met Gln Leu Ala Glu Gln Phe Lys Gln Lys
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gcg ctc ggg tta ctg caa aca gcc acc aaa caa gcg gag gct gct gct 4502
Ala Leu Gly Leu Leu Gln Thr Ala Thr Lys Gln Ala Glu Ala Ala Ala
1105 1110 1115

ccc gtg gtg gag tcc aag tgg cga gcc ctt gag aca ttc tgg gcg aag 4550
Pro Val Val Glu Ser Lys Trp Arg Ala Leu Glu Thr Phe Trp Ala Lys
1120 1125 1130

cac atg tgg aat ttc atc agc ggg ata cag tac tta gca ggc tta tcc 4598
His Met Trp Asn Phe Ile Ser Gly Ile Gln Tyr Leu Ala Gly Leu Ser
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Thr Leu Pro Gly Asn Pro Ala Ile Ala Ser Leu Met Ala Phe Thr Ala
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1200 1205 1210

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acc gag gac ctg gtc aat cta ctt cct gcc atc ctc gag gaa gct agt 4934
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Ser Lys Thr Tyr Asn Val Asp Lys His Val Pro Asp Ser Gly Ala Thr
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Tyr Ala His Thr Val Asn Arg Asn Trp Tyr Ser Asp Ala Asp Val Pro
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Phe Ala Ala Cys Leu Glu Pro Tyr Thr Ala Cys Asp Leu Ala Pro Pro
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                   Met Ser Glu Lys Tyr Ile Val Thr Trp Asp Met
                  1785                1790                1795

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Leu Gln Ile His Ala Arg Lys Leu Ala Ser Arg Leu Met Pro Ser Glu
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caa tgg aaa ggc att att gcc gta agc cgt ggc ggt ctg gta ccg ggt    8707
Gln Trp Lys Gly Ile Ile Ala Val Ser Arg Gly Gly Leu Val Pro Gly
          1815                1820                1825

gcg tta ctg gcg cgt gaa ctg ggt att cgt cat gtc gat acc gtt tgt    8755
Ala Leu Leu Ala Arg Glu Leu Gly Ile Arg His Val Asp Thr Val Cys
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att tcc agc tac gat cac gac aac cag cgc gag ctt aaa gtg ctg aaa    8803
Ile Ser Ser Tyr Asp His Asp Asn Gln Arg Glu Leu Lys Val Leu Lys
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Arg Ala Glu Gly Asp Gly Glu Gly Phe Ile Val Ile Asp Asp Leu Val
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gat acc ggt ggt act gcg gtt gcg att cgt gaa atg tat cca aaa gcg    8899
Asp Thr Gly Gly Thr Ala Val Ala Ile Arg Glu Met Tyr Pro Lys Ala
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cac ttt gtc acc atc ttc gca aaa ccg gct ggt cgt ccg ctg gtt gat    8947
His Phe Val Thr Ile Phe Ala Lys Pro Ala Gly Arg Pro Leu Val Asp
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Asp Tyr Val Val Asp Ile Pro Gln Asp Thr Trp Ile Glu Gln Pro Trp
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Asp Met Gly Val Val Phe Val Pro Pro Ile Ser Gly Arg
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       Phe Cys Glu Arg Met Ala Asn Glu Gly Lys Ile Val Ile Val
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gcc gca ctc gat ggg aca ttt caa cgt aaa ccg ttt aat aat att ttg    10280
Ala Ala Leu Asp Gly Thr Phe Gln Arg Lys Pro Phe Asn Asn Ile Leu
                1955                1960                1965

aat ctt att cca tta tct gaa atg gtg gta aaa cta act gct gtg tgt    10328
Asn Leu Ile Pro Leu Ser Glu Met Val Val Lys Leu Thr Ala Val Cys
            1970                1975                1980

atg aaa tgc ttt aag gag gct tcc ttt tct aaa cga ttg ggt gag gaa    10376
Met Lys Cys Phe Lys Glu Ala Ser Phe Ser Lys Arg Leu Gly Glu Glu
        1985                1990                1995

acc gag ata gaa ata ata gga ggt aat gat atg tat caa tcg gtg tgt    10424
Thr Glu Ile Glu Ile Ile Gly Gly Asn Asp Met Tyr Gln Ser Val Cys
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Arg Lys Cys Tyr Ile Asp Ser
2015                2020

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                        Met Ser Ile Gln His Phe Arg Val Ala Leu
                                    2025                2030

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Ile Pro Phe Phe Ala Ala Phe Cys Leu Pro Val Phe Ala His Pro Glu
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acg ctg gtg aaa gta aaa gat gct gaa gat cag ttg ggt gca cga gtg    12002
Thr Leu Val Lys Val Lys Asp Ala Glu Asp Gln Leu Gly Ala Arg Val
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Gly Tyr Ile Glu Leu Asp Leu Asn Ser Gly Lys Ile Leu Glu Ser Phe
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Arg Pro Glu Glu Arg Phe Pro Met Met Ser Thr Phe Lys Val Leu Leu
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Cys Gly Ala Val Leu Ser Arg Ile Asp Ala Gly Gln Glu Gln Leu Gly
                2100                2105                2110
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aca gaa aag cat ctt acg gat ggc atg aca gta aga gaa tta tgc agt 12242
Thr Glu Lys His Leu Thr Asp Gly Met Thr Val Arg Glu Leu Cys Ser
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Ala Ala Ile Thr Met Ser Asp Asn Thr Ala Ala Asn Leu Leu Leu Thr
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Tyr Lys Cys Val Thr Ile Lys Tyr Ser Asn Asp Asn Arg Tyr Gly Thr
35 40 45

Gly Leu Trp Thr His Asp Lys Asn Asn Phe Glu Ala Leu Glu Ala Thr
50 55 60

Lys Leu Cys Asp Val Leu Glu Ser Ile Thr Asp Phe Ser Val Ile Gly
65 70 75 80

Ile Asp Glu Gly Gln Phe Phe Pro Asp Ile Val Glu Met Gly Ile Pro
85 90 95

Gln Phe Met Ala Arg Val Cys Ala Cys Leu Trp Met Met Leu Leu Ile
100 105 110

Ala Gln Ala Glu Ala Ala Leu Glu Asn Leu Val Val Leu Asn Ala Ala
115 120 125

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Ser Val Ala Gly Ala His Gly Ile Leu Ser Phe Leu Val Phe Phe Cys
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Ala Ala Trp Tyr Ile Lys Gly Arg Leu Val Pro Gly Ala Ala Tyr Ala
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Leu Tyr Gly Val Trp Pro Leu Leu Leu Leu Leu Leu Ala Leu Pro Pro
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Arg Ala Tyr Ala Met Asp Arg Glu Met Ala Ala Ser Cys Gly Gly Ala
            180                 185                 190

Val Phe Val Gly Leu Val Leu Leu Thr Leu Ser Pro Tyr Tyr Lys Val
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Phe Leu Ala Arg Leu Ile Trp Trp Leu Gln Tyr Phe Thr Thr Arg Ala
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Glu Ala His Leu His Val Trp Ile Pro Pro Leu Asn Ala Arg Gly Gly
225                 230                 235                 240

Arg Asp Ala Ile Ile Leu Leu Met Cys Ala Val His Pro Glu Leu Ile
                245                 250                 255

Phe Asp Ile Thr Lys Leu Leu Ile Ala Ile Leu Gly Pro Leu Met Val
            260                 265                 270

Leu Gln Ala Gly Ile Thr Arg Val Pro Tyr Phe Val Arg Ala Gln Gly
        275                 280                 285

Leu Ile His Ala Cys Met Leu Val Arg Lys Val Ala Gly Gly His Tyr
    290                 295                 300

Val Gln Met Ala Phe Met Lys Leu Gly Ala Leu Thr Gly Thr Tyr Ile
305                 310                 315                 320

Tyr Asn His Leu Thr Pro Leu Arg Asp Trp Ala His Ala Gly Leu Arg
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Asp Leu Ala Val Ala Val Glu Pro Val Val Phe Ser Asp Met Glu Thr
            340                 345                 350

Lys Ile Ile Thr Trp Gly Ala Asp Thr Ala Ala Cys Gly Asp Ile Ile
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Leu Gly Leu Pro Val Ser Ala Arg Arg Gly Lys Glu Ile Leu Leu Gly
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Pro Ala Asp Ser Leu Glu Gly Arg Gly Trp Arg Leu Leu Ala Pro Ile
385                 390                 395                 400

Thr Ala Tyr Ser Gln Gln Thr Arg Gly Leu Leu Gly Cys Ile Ile Thr
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Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu Gly Glu Val Gln Val
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Val Ser Thr Ala Thr Gln Ser Phe Leu Ala Thr Cys Val Asn Gly Val
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Gly Ser Ser Asp Leu Tyr Leu Val Thr Arg His Ala Asp Val Ile Pro
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Val Ser Tyr Leu Lys Gly Ser Ala Gly Gly Pro Leu Leu Cys Pro Ser
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Gly His Ala Val Gly Ile Phe Arg Ala Ala Val Cys Thr Arg Gly Val
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Ala Lys Ala Val Asp Phe Val Pro Val Glu Ser Met Glu Thr Thr Met
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Arg Ser Pro Val Phe Thr Asp Asn Ser Ser Pro Pro Ala Val Pro Gln
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Ser Phe Gln Val Ala His Leu His Ala Pro Thr Gly Ser Gly Lys Ser
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Thr Lys Val Pro Ala Ala Tyr Ala Ala Gln Gly Tyr Lys Val Leu Val
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625                 630                 635                 640

Lys Ala His Gly Ile Asp Pro Asn Ile Arg Thr Gly Val Arg Thr Ile
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Thr Thr Gly Ala Pro Val Thr Tyr Ser Thr Tyr Gly Lys Phe Leu Ala
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Asp Gly Gly Cys Ser Gly Gly Ala Tyr Asp Ile Ile Ile Cys Asp Glu
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Cys His Ser Thr Asp Ser Thr Thr Ile Leu Gly Ile Gly Thr Val Leu
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Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Val Val Leu Ala Thr Ala
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Thr Pro Pro Gly Ser Val Thr Val Pro His Pro Asn Ile Glu Glu Val
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Ala Leu Ser Asn Thr Gly Glu Ile Pro Phe Tyr Gly Lys Ala Ile Pro
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Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile Glu Thr Thr Thr Val
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Pro Gln Asp Ala Val Ser Arg Ser Gln Arg Arg Gly Arg Thr Gly Arg
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Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys Tyr Asp Ala Gly Cys
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Tyr Leu Asn Thr Pro Gly Leu Pro Val Cys Gln Asp His Leu Glu Phe
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Trp Glu Ser Val Phe Thr Gly Leu Thr His Ile Asp Ala His Phe Leu
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Ser Gln Thr Lys Gln Ala Gly Asp Asn Phe Pro Tyr Leu Val Ala Tyr
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Gln Met Trp Lys Cys Leu Ile Arg Leu Lys Pro Thr Leu His Gly Pro
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His Thr Val Asn Arg Asn Trp Tyr Ser Asp Ala Asp Val Pro Ala Ser
1460 1465 1470

Ala Arg Gln Glu Gly Cys Gln Asp Ile Ala Thr Gln Leu Ile Ser Asn
1475 1480 1485

Met Asp Ile Asp Val Ile Leu Gly Gly Gly Arg Lys Tyr Met Phe Pro
1490 1495 1500

Met Gly Thr Pro Asp Pro Glu Tyr Pro Asp Asp Tyr Ser Gln Gly Gly
505 1510 1515 1520

Thr Arg Leu Asp Gly Lys Asn Leu Val Gln Glu Trp Leu Ala Lys Arg
1525 1530 1535

Gln Gly Ala Arg Tyr Val Trp Asn Arg Thr Glu Leu Met Gln Ala Ser
1540 1545 1550

Leu Asp Pro Ser Val Thr His Leu Met Gly Leu Phe Glu Pro Gly Asp
1555 1560 1565

Met Lys Tyr Glu Ile His Arg Asp Ser Thr Leu Asp Pro Ser Leu Met
1570 1575 1580

Glu Met Thr Glu Ala Ala Leu Arg Leu Leu Ser Arg Asn Pro Arg Gly
585 1590 1595 1600

Phe Phe Leu Phe Val Glu Gly Gly Arg Ile Asp His Gly His His Glu
1605 1610 1615

Ser Arg Ala Tyr Arg Ala Leu Thr Glu Thr Ile Met Phe Asp Asp Ala
1620 1625 1630

Ile Glu Arg Ala Gly Gln Leu Thr Ser Glu Glu Asp Thr Leu Ser Leu
1635 1640 1645

Val Thr Ala Asp His Ser His Val Phe Ser Phe Gly Gly Tyr Pro Leu
1650 1655 1660

Arg Gly Ser Cys Ile Phe Gly Leu Ala Pro Gly Lys Ala Arg Asp Arg
665 1670 1675 1680

Lys Ala Tyr Thr Val Leu Leu Tyr Gly Asn Gly Pro Gly Tyr Val Leu
1685 1690 1695

Lys Asp Gly Ala Arg Pro Asp Val Thr Glu Ser Glu Ser Gly Ser Pro
1700 1705 1710

Glu Tyr Arg Gln Gln Ser Ala Val Pro Leu Asp Glu Glu Thr His Ala
1715 1720 1725

Gly Glu Asp Val Ala Val Phe Ala Arg Gly Pro Gln Ala His Leu Val
1730 1735 1740

His Gly Val Gln Glu Gln Thr Phe Ile Ala His Val Met Ala Phe Ala
745 1750 1755 1760

Ala Cys Leu Glu Pro Tyr Thr Ala Cys Asp Leu Ala Pro Pro Ala Gly
1765 1770 1775

Thr Thr Asp Ala Ala His Pro Gly Met Ser Glu Lys Tyr Ile Val Thr
1780 785 1790

Trp Asp Met Leu Gln Ile His Ala Arg Lys Leu Ala Ser Arg Leu Met
1795 1800 1805

Pro Ser Glu Gln Trp Lys Gly Ile Ile Ala Val Ser Arg Gly Gly Leu
1810 1815 1820

Val Pro Gly Ala Leu Leu Ala Arg Glu Leu Gly Ile Arg His Val Asp
825 1830 1835 1840

Thr Val Cys Ile Ser Ser Tyr Asp His Asp Asn Gln Arg Glu Leu Lys
1845 1850 1855

Val Leu Lys Arg Ala Glu Gly Asp Gly Glu Gly Phe Ile Val Ile Asp
1860 1865 1870

Asp Leu Val Asp Thr Gly Gly Thr Ala Val Ala Ile Arg Glu Met Tyr
1875 1880 1885

Pro Lys Ala His Phe Val Thr Ile Phe Ala Lys Pro Ala Gly Arg Pro
1890 1895 1900

Leu Val Asp Asp Tyr Val Val Asp Ile Pro Gln Asp Thr Trp Ile Glu
905 1910 1915 1920

Gln Pro Trp Asp Met Gly Val Val Phe Val Pro Pro Ile Ser Gly Arg
1925 1930 1935

Phe Cys Glu Arg Met Ala Asn Glu Gly Lys Ile Val Ile Val Ala Ala
1940 1945 1950

Leu Asp Gly Thr Phe Gln Arg Lys Pro Phe Asn Asn Ile Leu Asn Leu
1955 1960 1965

Ile Pro Leu Ser Glu Met Val Val Lys Leu Thr Ala Val Cys Met Lys
1970 1975 1980

Cys Phe Lys Glu Ala Ser Phe Ser Lys Arg Leu Gly Glu Glu Thr Glu
985 1990 1995 2000

Ile Glu Ile Ile Gly Gly Asn Asp Met Tyr Gln Ser Val Cys Arg Lys
2005 2010 2015

Cys Tyr Ile Asp Ser Met Ser Ile Gln His Phe Arg Val Ala Leu Ile
2020 2025 2030

Pro Phe Phe Ala Ala Phe Cys Leu Pro Val Phe Ala His Pro Glu Thr
2035 2040 2045

Leu Val Lys Val Lys Asp Ala Glu Asp Gln Leu Gly Ala Arg Val Gly
2050 2055 2060

Tyr Ile Glu Leu Asp Leu Asn Ser Gly Lys Ile Leu Glu Ser Phe Arg
065 2070 2075 208

Pro Glu Glu Arg Phe Pro Met Met Ser Thr Phe Lys Val Leu Leu Cys
2085 2090 2095

Gly Ala Val Leu Ser Arg Ile Asp Ala Gly Gln Glu Gln Leu Gly Arg
2100 2105 2110

Arg Ile His Tyr Ser Gln Asn Asp Leu Val Glu Tyr Ser Pro Val Thr
2115 2120 2125

Glu Lys His Leu Thr Asp Gly Met Thr Val Arg Glu Leu Cys Ser Ala
2130 2135 2140

Ala Ile Thr Met Ser Asp Asn Thr Ala Ala Asn Leu Leu Leu Thr Thr
145 2150 2155 216

Ile Gly Gly Pro Lys Glu Leu Thr Ala Phe Leu His Asn Met Gly Asp
2165 2170 2175

His Val Thr Arg Leu Asp Arg Trp Glu Pro Glu Leu Asn Glu Ala Ile
2180 2185 2190

Pro Asn Asp Glu Arg Asp Thr Thr Met Pro Val Ala Met Ala Thr Thr
2195 2200 2205

Leu Arg Lys Leu Leu Thr Gly Glu Leu Leu Thr Leu Ala Ser Arg Gln
2210 2215 2220

Gln Leu Ile Asp Trp Met Glu Ala Asp Lys Val Ala Gly Pro Leu Leu
225 2230 2235 224

Arg Ser Ala Leu Pro Ala Gly Trp Phe Ile Ala Asp Lys Ser Gly Ala
2245 2250 2255

Gly Glu Arg Gly Ser Arg Gly Ile Ile Ala Ala Leu Gly Pro Asp Gly
2260 2265 2270

Lys Pro Ser Arg Ile Val Val Ile Tyr Thr Thr Gly Ser Gln Ala Thr
2275 2280 2285

Met Asp Glu Arg Asn Arg Gln Ile Ala Glu Ile Gly Ala Ser Leu Ile
2290 2295 2300

Lys His Trp
305

<210> 10
<211> 92
<212> PRT
<213> Artificial Sequence

<400> 10
Met Asn Gly Gly His Ile Gln Leu Ile Ile Gly Pro Met Phe Ser Gly
1 5 10 15

Lys Ser Thr Glu Leu Ile Arg Arg Val Arg Arg Tyr Gln Ile Ala Gln
20 25 30

Tyr Lys Cys Val Thr Ile Lys Tyr Ser Asn Asp Asn Arg Tyr Gly Thr
35 40 45

Gly Leu Trp Thr His Asp Lys Asn Asn Phe Glu Ala Leu Glu Ala Thr
50 55 60

Lys Leu Cys Asp Val Leu Glu Ser Ile Thr Asp Phe Ser Val Ile Gly
65 70 75 80

Ile Asp Glu Gly Gln Phe Phe Pro Asp Ile Val Glu
85 90

<210> 11
<211> 1692
<212> PRT
<213> Artificial Sequence

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Met Gly Ile Pro Gln Phe Met Ala Arg Val Cys Ala Cys Leu Trp Met
1 5 10 15

Met Leu Leu Ile Ala Gln Ala Glu Ala Ala Leu Glu Asn Leu Val Val
20 25 30

Leu Asn Ala Ala Ser Val Ala Gly Ala His Gly Ile Leu Ser Phe Leu
35 40 45

Val Phe Phe Cys Ala Ala Trp Tyr Ile Lys Gly Arg Leu Val Pro Gly
50 55 60

Ala Ala Tyr Ala Leu Tyr Gly Val Trp Pro Leu Leu Leu Leu Leu Leu
65 70 75 80

Ala Leu Pro Pro Arg Ala Tyr Ala Met Asp Arg Glu Met Ala Ala Ser
85 90 95

Cys Gly Gly Ala Val Phe Val Gly Leu Val Leu Leu Thr Leu Ser Pro
100 105 110

Tyr Tyr Lys Val Phe Leu Ala Arg Leu Ile Trp Trp Leu Gln Tyr Phe
115 120 125

Thr Thr Arg Ala Glu Ala His Leu His Val Trp Ile Pro Pro Leu Asn
130 135 140

Ala Arg Gly Gly Arg Asp Ala Ile Ile Leu Leu Met Cys Ala Val His
145 150 155 160

Pro Glu Leu Ile Phe Asp Ile Thr Lys Leu Leu Ile Ala Ile Leu Gly
165 170 175

Pro Leu Met Val Leu Gln Ala Gly Ile Thr Arg Val Pro Tyr Phe Val
180 185 190

Arg Ala Gln Gly Leu Ile His Ala Cys Met Leu Val Arg Lys Val Ala
195 200 205

Gly Gly His Tyr Val Gln Met Ala Phe Met Lys Leu Gly Ala Leu Thr
    210   215   220

Gly Thr Tyr Ile Tyr Asn His Leu Thr Pro Leu Arg Asp Trp Ala His
225   230   235   240

Ala Gly Leu Arg Asp Leu Ala Val Ala Val Glu Pro Val Val Phe Ser
                245   250   255

Asp Met Glu Thr Lys Ile Ile Thr Trp Gly Ala Asp Thr Ala Ala Cys
            260   265   270

Gly Asp Ile Ile Leu Gly Leu Pro Val Ser Ala Arg Arg Gly Lys Glu
        275   280   285

Ile Leu Leu Gly Pro Ala Asp Ser Leu Glu Gly Arg Gly Trp Arg Leu
    290   295   300

Leu Ala Pro Ile Thr Ala Tyr Ser Gln Gln Thr Arg Gly Leu Leu Gly
305   310   315   320

Cys Ile Ile Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu Gly
                325   330   335

Glu Val Gln Val Val Ser Thr Ala Thr Gln Ser Phe Leu Ala Thr Cys
            340   345   350

Val Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly Ser Lys Thr
        355   360   365

Leu Ala Gly Pro Lys Gly Pro Ile Thr Gln Met Tyr Thr Asn Val Asp
    370   375   380

Gln Asp Leu Val Gly Trp Gln Ala Pro Pro Gly Ala Arg Ser Leu Thr
385   390   395   400

Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr Leu Val Thr Arg His Ala
                405   410   415

Asp Val Ile Pro Val Arg Arg Arg Gly Asp Ser Arg Gly Ser Leu Leu
            420   425   430

Ser Pro Arg Pro Val Ser Tyr Leu Lys Gly Ser Ala Gly Gly Pro Leu
        435   440   445

Leu Cys Pro Ser Gly His Ala Val Gly Ile Phe Arg Ala Ala Val Cys
    450   455   460

Thr Arg Gly Val Ala Lys Ala Val Asp Phe Val Pro Val Glu Ser Met
465   470   475   480

Glu Thr Thr Met Arg Ser Pro Val Phe Thr Asp Asn Ser Ser Pro Pro
                485   490   495

Ala Val Pro Gln Ser Phe Gln Val Ala His Leu His Ala Pro Thr Gly
            500   505   510

Ser Gly Lys Ser Thr Lys Val Pro Ala Ala Tyr Ala Ala Gln Gly Tyr
        515   520   525

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Lys Val Leu Val Leu Asn Pro Ser Val Ala Ala Thr Leu Gly Phe Gly
    530                 535                 540

Ala Tyr Met Ser Lys Ala His Gly Ile Asp Pro Asn Ile Arg Thr Gly
545                 550                 555                 560

Val Arg Thr Ile Thr Thr Gly Ala Pro Val Thr Tyr Ser Thr Tyr Gly
                565                 570                 575

Lys Phe Leu Ala Asp Gly Gly Cys Ser Gly Gly Ala Tyr Asp Ile Ile
            580                 585                 590

Ile Cys Asp Glu Cys His Ser Thr Asp Ser Thr Thr Ile Leu Gly Ile
        595                 600                 605

Gly Thr Val Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Val Val
    610                 615                 620

Leu Ala Thr Ala Thr Pro Pro Gly Ser Val Thr Val Pro His Pro Asn
625                 630                 635                 640

Ile Glu Glu Val Ala Leu Ser Asn Thr Gly Glu Ile Pro Phe Tyr Gly
                645                 650                 655

Lys Ala Ile Pro Ile Glu Ala Ile Arg Gly Gly Arg His Leu Ile Phe
            660                 665                 670

Cys His Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Ser Gly
        675                 680                 685

Leu Gly Ile Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val
    690                 695                 700

Ile Pro Thr Ile Gly Asp Val Val Val Val Ala Thr Asp Ala Leu Met
705                 710                 715                 720

Thr Gly Tyr Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Thr Cys
                725                 730                 735

Val Thr Gln Thr Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile Glu
            740                 745                 750

Thr Thr Thr Val Pro Gln Asp Ala Val Ser Arg Ser Gln Arg Arg Gly
        755                 760                 765

Arg Thr Gly Arg Gly Arg Arg Gly Ile Tyr Arg Phe Val Thr Pro Gly
    770                 775                 780

Glu Arg Pro Ser Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys Tyr
785                 790                 795                 800

Asp Ala Gly Cys Ala Trp Tyr Glu Leu Thr Pro Ala Glu Thr Ser Val
                805                 810                 815

Arg Leu Arg Ala Tyr Leu Asn Thr Pro Gly Leu Pro Val Cys Gln Asp
            820                 825                 830

His Leu Glu Phe Trp Glu Ser Val Phe Thr Gly Leu Thr His Ile Asp
        835                 840                 845
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Ala His Phe Leu Ser Gln Thr Lys Gln Ala Gly Asp Asn Phe Pro Tyr
850 855 860

Leu Val Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro Pro
865 870 875 880

Pro Ser Trp Asp Gln Met Trp Lys Cys Leu Ile Arg Leu Lys Pro Thr
885 890 895

Leu His Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln Asn
900 905 910

Glu Val Thr Leu Thr His Pro Ile Thr Lys Tyr Ile Met Ala Cys Met
915 920 925

Ser Ala Asp Leu Glu Val Val Thr Ser Thr Trp Val Leu Val Gly Gly
930 935 940

Val Leu Ala Ala Leu Ala Ala Tyr Cys Leu Thr Thr Gly Ser Val Val
945 950 955 960

Ile Val Gly Arg Ile Ile Leu Ser Gly Arg Pro Ala Ile Val Pro Asp
965 970 975

Arg Glu Leu Leu Tyr Gln Glu Phe Asp Glu Met Glu Glu Cys Ala Ser
980 985 990

His Leu Pro Tyr Ile Glu Gln Gly Met Gln Leu Ala Glu Gln Phe Lys
995 1000 1005

Gln Lys Ala Leu Gly Leu Leu Gln Thr Ala Thr Lys Gln Ala Glu Ala
1010 1015 1020

Ala Ala Pro Val Val Glu Ser Lys Trp Arg Ala Leu Glu Thr Phe Trp
025 1030 1035 1040

Ala Lys His Met Trp Asn Phe Ile Ser Gly Ile Gln Tyr Leu Ala Gly
1045 1050 1055

Leu Ser Thr Leu Pro Gly Asn Pro Ala Ile Ala Ser Leu Met Ala Phe
1060 1065 1070

Thr Ala Ser Ile Thr Ser Pro Leu Thr Thr Gln Ser Thr Leu Leu Phe
1075 1080 1085

Asn Ile Leu Gly Gly Trp Val Ala Ala Gln Leu Ala Pro Pro Ser Ala
1090 1095 1100

Ala Ser Ala Phe Val Gly Ala Gly Ile Ala Gly Ala Ala Val Gly Ser
105 1110 1115 1120

Ile Gly Leu Gly Lys Val Leu Val Asp Ile Leu Ala Gly Tyr Gly Ala
1125 1130 1135

Gly Val Ala Gly Ala Leu Val Ala Phe Lys Val Met Ser Gly Glu Met
1140 1145 1150

Pro Ser Thr Glu Asp Leu Val Asn Leu Leu Pro Ala Ile Leu Glu Glu
1155 1160 1165

Ala Ser Glu Asp Val Val Cys Cys Ser Met Ser Tyr Thr Trp Thr Gly
1170 1175 1180

Ala Leu Glu Leu Leu Leu Leu Leu Leu Leu Gly Leu Arg Leu Gln Leu
185 1190 1195 1200

Ser Leu Gly Ile Ile Pro Val Glu Glu Glu Asn Pro Asp Phe Trp Asn
1205 1210 1215

Arg Glu Ala Ala Glu Ala Leu Gly Ala Ala Lys Lys Leu Gln Pro Ala
1220 1225 1230

Gln Thr Ala Ala Lys Asn Leu Ile Ile Phe Leu Gly Asp Gly Met Gly
1235 1240 1245

Val Ser Thr Val Thr Ala Ala Arg Ile Leu Lys Gly Gln Lys Lys Asp
1250 1255 1260

Lys Leu Gly Pro Glu Ile Pro Leu Ala Met Asp Arg Phe Pro Tyr Val
265 1270 1275 1280

Ala Leu Ser Lys Thr Tyr Asn Val Asp Lys His Val Pro Asp Ser Gly
1285 1290 1295

Ala Thr Ala Thr Ala Tyr Leu Cys Gly Val Lys Gly Asn Phe Gln Thr
1300 1305 1310

Ile Gly Leu Ser Ala Ala Ala Arg Phe Asn Gln Cys Asn Thr Thr Arg
1315 1320 1325

Gly Asn Glu Val Ile Ser Val Met Asn Arg Ala Lys Lys Ala Gly Lys
1330 1335 1340

Ser Val Gly Val Val Thr Thr Thr Arg Val Gln His Ala Ser Pro Ala
345 1350 1355 1360

Gly Thr Tyr Ala His Thr Val Asn Arg Asn Trp Tyr Ser Asp Ala Asp
1365 1370 1375

Val Pro Ala Ser Ala Arg Gln Glu Gly Cys Gln Asp Ile Ala Thr Gln
1380 1385 1390

Leu Ile Ser Asn Met Asp Ile Asp Val Ile Leu Gly Gly Gly Arg Lys
1395 1400 1405

Tyr Met Phe Pro Met Gly Thr Pro Asp Pro Glu Tyr Pro Asp Asp Tyr
1410 1415 1420

Ser Gln Gly Gly Thr Arg Leu Asp Gly Lys Asn Leu Val Gln Glu Trp
425 1430 1435 1440

Leu Ala Lys Arg Gln Gly Ala Arg Tyr Val Trp Asn Arg Thr Glu Leu
1445 1450 1455

Met Gln Ala Ser Leu Asp Pro Ser Val Thr His Leu Met Gly Leu Phe
1460 1465 1470

Glu Pro Gly Asp Met Lys Tyr Glu Ile His Arg Asp Ser Thr Leu Asp
1475 1480 1485

Pro Ser Leu Met Glu Met Thr Glu Ala Ala Leu Arg Leu Leu Ser Arg
1490 1495 1500

Asn Pro Arg Gly Phe Phe Leu Phe Val Glu Gly Gly Arg Ile Asp His
505 1510 1515 1520

Gly His His Glu Ser Arg Ala Tyr Arg Ala Leu Thr Glu Thr Ile Met
1525 1530 1535

Phe Asp Asp Ala Ile Glu Arg Ala Gly Gln Leu Thr Ser Glu Glu Asp
1540 1545 1550

Thr Leu Ser Leu Val Thr Ala Asp His Ser His Val Phe Ser Phe Gly
1555 1560 1565

Gly Tyr Pro Leu Arg Gly Ser Cys Ile Phe Gly Leu Ala Pro Gly Lys
1570 1575 1580

Ala Arg Asp Arg Lys Ala Tyr Thr Val Leu Leu Tyr Gly Asn Gly Pro
585 1590 1595 1600

Gly Tyr Val Leu Lys Asp Gly Ala Arg Pro Asp Val Thr Glu Ser Glu
1605 1610 1615

Ser Gly Ser Pro Glu Tyr Arg Gln Gln Ser Ala Val Pro Leu Asp Glu
1620 1625 1630

Glu Thr His Ala Gly Glu Asp Val Ala Val Phe Ala Arg Gly Pro Gln
1635 1640 1645

Ala His Leu Val His Gly Val Gln Glu Gln Thr Phe Ile Ala His Val
1650 1655 1660

Met Ala Phe Ala Ala Cys Leu Glu Pro Tyr Thr Ala Cys Asp Leu Ala
665 1670 1675 1680

Pro Pro Ala Gly Thr Thr Asp Ala Ala His Pro Gly
1685 1690

<210> 12
<211> 152
<212> PRT
<213> Artificial Sequence

<400> 12
Met Ser Glu Lys Tyr Ile Val Thr Trp Asp Met Leu Gln Ile His Ala
1 5 10 15

Arg Lys Leu Ala Ser Arg Leu Met Pro Ser Glu Gln Trp Lys Gly Ile
20 25 30

Ile Ala Val Ser Arg Gly Gly Leu Val Pro Gly Ala Leu Leu Ala Arg
35 40 45

Glu Leu Gly Ile Arg His Val Asp Thr Val Cys Ile Ser Ser Tyr Asp
50 55 60

His Asp Asn Gln Arg Glu Leu Lys Val Leu Lys Arg Ala Glu Gly Asp
65 70 75 80

Gly Glu Gly Phe Ile Val Ile Asp Asp Leu Val Asp Thr Gly Gly Thr
85 90 95

Ala Val Ala Ile Arg Glu Met Tyr Pro Lys Ala His Phe Val Thr Ile
100 105 110

Phe Ala Lys Pro Ala Gly Arg Pro Leu Val Asp Asp Tyr Val Val Asp
115 120 125

Ile Pro Gln Asp Thr Trp Ile Glu Gln Pro Trp Asp Met Gly Val Val
130 135 140

Phe Val Pro Pro Ile Ser Gly Arg
145 150

<210> 13
<211> 85
<212> PRT
<213> Artificial Sequence

<400> 13
Phe Cys Glu Arg Met Ala Asn Glu Gly Lys Ile Val Ile Val Ala Ala
1 5 10 15

Leu Asp Gly Thr Phe Gln Arg Lys Pro Phe Asn Asn Ile Leu Asn Leu
20 25 30

Ile Pro Leu Ser Glu Met Val Val Lys Leu Thr Ala Val Cys Met Lys
35 40 45

Cys Phe Lys Glu Ala Ser Phe Ser Lys Arg Leu Gly Glu Glu Thr Glu
50 55 60

Ile Glu Ile Ile Gly Gly Asn Asp Met Tyr Gln Ser Val Cys Arg Lys
65 70 75 80

Cys Tyr Ile Asp Ser
85

<210> 14
<211> 286
<212> PRT
<213> Artificial Sequence

<400> 14
Met Ser Ile Gln His Phe Arg Val Ala Leu Ile Pro Phe Phe Ala Ala
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Phe Cys Leu Pro Val Phe Ala His Pro Glu Thr Leu Val Lys Val Lys
20 25 30

Asp Ala Glu Asp Gln Leu Gly Ala Arg Val Gly Tyr Ile Glu Leu Asp
35 40 45

Leu Asn Ser Gly Lys Ile Leu Glu Ser Phe Arg Pro Glu Glu Arg Phe
50 55 60

Pro Met Met Ser Thr Phe Lys Val Leu Leu Cys Gly Ala Val Leu Ser
65 70 75 80

Arg Ile Asp Ala Gly Gln Glu Gln Leu Gly Arg Arg Ile His Tyr Ser
85 90 95

Gln Asn Asp Leu Val Glu Tyr Ser Pro Val Thr Glu Lys His Leu Thr
100 105 110

Asp Gly Met Thr Val Arg Glu Leu Cys Ser Ala Ala Ile Thr Met Ser
115 120 125

Asp Asn Thr Ala Ala Asn Leu Leu Leu Thr Thr Ile Gly Gly Pro Lys
130 135 140

Glu Leu Thr Ala Phe Leu His Asn Met Gly Asp His Val Thr Arg Leu
145 150 155 160

Asp Arg Trp Glu Pro Glu Leu Asn Glu Ala Ile Pro Asn Asp Glu Arg
165 170 175

Asp Thr Thr Met Pro Val Ala Met Ala Thr Thr Leu Arg Lys Leu Leu
180 185 190

Thr Gly Glu Leu Leu Thr Leu Ala Ser Arg Gln Gln Leu Ile Asp Trp
195 200 205

Met Glu Ala Asp Lys Val Ala Gly Pro Leu Leu Arg Ser Ala Leu Pro
210 215 220

Ala Gly Trp Phe Ile Ala Asp Lys Ser Gly Ala Gly Glu Arg Gly Ser
225 230 235 240

Arg Gly Ile Ile Ala Ala Leu Gly Pro Asp Gly Lys Pro Ser Arg Ile
245 250 255

Val Val Ile Tyr Thr Thr Gly Ser Gln Ala Thr Met Asp Glu Arg Asn
260 265 270

Arg Gln Ile Ala Glu Ile Gly Ala Ser Leu Ile Lys His Trp
275 280 285

<210> 15
<211> 13910
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: plasmid phcap 4

<220>
<221> CDS
<222> (497)..(772)

<220>
<221> CDS
<222> (1425)..(6500)

<220>
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<222> (8579)..(9034)

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<221> CDS
<222> (10191)..(10445)

<220>
<221> CDS
<222> (11877)..(12734)

<220>
<221> misc_feature
<222> (1)..(774)
<223> Vaccinia Virus thymidine Kinase gene recombination site

<220>
<221> promoter
<222> (794)..(816)
<223> T7 promoter

<220>
<221> misc_feature
<222> (846)..(1424)
<223> EMC/Internal Ribosome Entry Site (IRES)

<220>
<221> misc_feature
<222> (1426)..(1437)
<223> MCS (Multiple Cloning Site)

<220>
<221> misc_feature
<222> (1446)..(2318)
<223> HCV E2/ NS2 domain

<220>
<221> misc_feature
<222> (2319)..(4231)
<223> HCV NS3 Domain containing the serine protease and helicase enzymes

<220>
<221> misc_feature
<222> (4203)..(4260)
<223> HCV NS3-NS4A cleavage site

<220>
<221> misc_feature
<222> (4375)..(4424)
<223> HCV NS4A-4B clevage site

<220>
<221> misc_feature
<222> (4233)..(4394)
<223> HCV NS4A domain

<220>
<221> misc_feature
<222> (4395)..(4919)
<223> HCV NS4B Domain

<220>

<221> misc_feature
<222> (4920)..(4991)
<223> HCV NS5A-NS5B cleavage site

<220>
<221> misc_feature
<222> (4992)..(6501)
<223> SEAP Protein

<220>
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<222> (7915)..(7945)
<223> MCS (Multiple Cloning Site)

<220>
<221> terminator
<222> (7938)..(8078)
<223> term T7

<220>
<221> promoter
<222> (8080)..(8365)
<223> Vacinina virus promoter; early/late promoter

<220>
<221> misc_feature
<222> (8560)..(11317)
<223> E. coli gpt; for selection of recombinants

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<221> misc_feature
<222> (11318)..(13909)
<223> remaining DNA from 3' end of Tropix pCMV/SEAP
plasmid

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tgatgattca ttttttaagt atttggctag tcaagatgat gaatcttcat tatctgatat 120

attgcaaatc actcaatatc tagactttct gttattatta ttgatccaat caaaaaataa 180

attagaagcc gtgggtcatt gttatgaatc tctttcagag gaatacagac aattgacaaa 240

attcacagac tttcaagatt ttaaaaaact gtttaacaag gtccctattg ttacagatgg 300

aagggtcaaa cttaataaag gatatttgtt cgactttgtg attagtttga tgcgattcaa 360

aaaagaatcc tctctagcta ccaccgcaat agatcctgtt agatacatag atcctcgtcg 420

caatatcgca ttttctaacg tgatggatat attaaagtcg aataaagtga acaataatta 480

attctttatt gtcatc atg aac ggc gga cat att cag ttg ata atc ggc ccc 532
Met Asn Gly Gly His Ile Gln Leu Ile Ile Gly Pro
1 5 10

atg ttt tca ggt aaa agt aca gaa tta att aga cga gtt aga cgt tat 580
Met Phe Ser Gly Lys Ser Thr Glu Leu Ile Arg Arg Val Arg Arg Tyr
15 20 25

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caa ata gct caa tat aaa tgc gtg act ata aaa tat tct aac gat aat    628
Gln Ile Ala Gln Tyr Lys Cys Val Thr Ile Lys Tyr Ser Asn Asp Asn
     30                  35                  40

aga tac gga acg gga cta tgg acg cat gat aag aat aat ttt gaa gca    676
Arg Tyr Gly Thr Gly Leu Trp Thr His Asp Lys Asn Asn Phe Glu Ala
 45                  50                  55                  60

ttg gaa gca act aaa cta tgt gat gtc ttg gaa tca att aca gat ttc    724
Leu Glu Ala Thr Lys Leu Cys Asp Val Leu Glu Ser Ile Thr Asp Phe
                 65                  70                  75

tcc gtg ata ggt atc gat gaa gga cag ttc ttt cca gac att gtt gaa    772
Ser Val Ile Gly Ile Asp Glu Gly Gln Phe Phe Pro Asp Ile Val Glu
             80                  85                  90

ttgatctcga tcccgcgaaa ttaatacgac tcactatagg gagaccacaa cggtttccct 832

ctagcgggat caattccgcc cctctccctc ccccccccct aacgttactg gccgaagccg 892

cttggaataa ggccggtgtg cgtttgtcta tatgttattt tccaccatat tgccgtcttt 952

tggcaatgtg agggcccgga aacctggccc tgtcttcttg acgagcattc ctaggggtct 1012

ttcccctctc gccaaaggaa tgcaaggtct gttgaatgtc gtgaaggaag cagttcctct 1072

ggaagcttct tgaagacaaa caacgtctgt agcgaccctt tgcaggcagc ggaacccccc 1132

acctggcgac aggtgcctct gcggccaaaa gccacgtgta taagatacac ctgcaaaggc 1192

ggcacaaccc cagtgccacg ttgtgagttg gatagttgtg gaaagagtca aatggctctc 1252

ctcaagcgta ttcaacaagg ggctgaagga tgcccagaag gtaccccatt gtatgggatc 1312

tgatctgggg cctcggtgca catgctttac atgtgtttag tcgaggttaa aaaacgtcta 1372

ggccccccga accacgggga cgtggttttc ctttgaaaaa cacgataata cc atg gga 1430
                                                          Met Gly

att ccc caa ttc atg gca cgt gtc tgt gcc tgc ttg tgg atg atg ctg    1478
Ile Pro Gln Phe Met Ala Arg Val Cys Ala Cys Leu Trp Met Met Leu
 95                 100                 105                 110

ctg ata gcc cag gcc gag gcc gcc ttg gag aac ctg gtg gtc ctc aat    1526
Leu Ile Ala Gln Ala Glu Ala Ala Leu Glu Asn Leu Val Val Leu Asn
                115                 120                 125

gcg gcg tct gtg gcc ggc gca cat ggc atc ctc tcc ttc ctt gtg ttc    1574
Ala Ala Ser Val Ala Gly Ala His Gly Ile Leu Ser Phe Leu Val Phe
            130                 135                 140

ttc tgt gcc gcc tgg tac atc aaa ggc agg ctg gtc cct ggg gcg gca    1622
Phe Cys Ala Ala Trp Tyr Ile Lys Gly Arg Leu Val Pro Gly Ala Ala
        145                 150                 155

tat gct ctt tat ggc gtg tgg ccg ctg ctc ctg ctc ttg ctg gca tta    1670
Tyr Ala Leu Tyr Gly Val Trp Pro Leu Leu Leu Leu Leu Leu Ala Leu
    160                 165                 170
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cca ccg cga gct tac gcc atg gac cgg gag atg gct gca tcg tgc gga 1718
Pro Pro Arg Ala Tyr Ala Met Asp Arg Glu Met Ala Ala Ser Cys Gly
175 180 185 190

ggc gcg gtt ttt gtg ggt ctg gta ctc ctg act ttg tca cca tac tac 1766
Gly Ala Val Phe Val Gly Leu Val Leu Leu Thr Leu Ser Pro Tyr Tyr
195 200 205

aag gtg ttc ctc gct agg ctc ata tgg tgg tta caa tat ttt acc acc 1814
Lys Val Phe Leu Ala Arg Leu Ile Trp Trp Leu Gln Tyr Phe Thr Thr
210 215 220

aga gcc gag gcg cac tta cat gtg tgg atc ccc ccc ctc aac gct cgg 1862
Arg Ala Glu Ala His Leu His Val Trp Ile Pro Pro Leu Asn Ala Arg
225 230 235

gga ggc cgc gat gcc atc atc ctc ctc atg tgc gca gtc cat cca gag 1910
Gly Gly Arg Asp Ala Ile Ile Leu Leu Met Cys Ala Val His Pro Glu
240 245 250

cta atc ttt gac atc acc aaa ctt cta att gcc ata ctc ggt ccg ctc 1958
Leu Ile Phe Asp Ile Thr Lys Leu Leu Ile Ala Ile Leu Gly Pro Leu
255 260 265 270

atg gtg ctc caa gct ggc ata acc aga gtg ccg tac ttc gtg cgc gct 2006
Met Val Leu Gln Ala Gly Ile Thr Arg Val Pro Tyr Phe Val Arg Ala
275 280 285

caa ggg ctc att cat gca tgc atg tta gtg cgg aag gtc gct ggg ggt 2054
Gln Gly Leu Ile His Ala Cys Met Leu Val Arg Lys Val Ala Gly Gly
290 295 300

cat tat gtc caa atg gcc ttc atg aag ctg ggc gcg ctg aca ggc acg 2102
His Tyr Val Gln Met Ala Phe Met Lys Leu Gly Ala Leu Thr Gly Thr
305 310 315

tac att tac aac cat ctt acc ccg cta cgg gat tgg gcc cac gcg ggc 2150
Tyr Ile Tyr Asn His Leu Thr Pro Leu Arg Asp Trp Ala His Ala Gly
320 325 330

cta cga gac ctt gcg gtg gca gtg gag ccc gtc gtc ttc tcc gac atg 2198
Leu Arg Asp Leu Ala Val Ala Val Glu Pro Val Val Phe Ser Asp Met
335 340 345 350

gag acc aag atc atc acc tgg gga gca gac acc gcg gcg gct ggg gac 2246
Glu Thr Lys Ile Ile Thr Trp Gly Ala Asp Thr Ala Ala Ala Gly Asp
355 360 365

atc atc ttg ggt ctg ccc gtc tcc gcc cga agg gga aag gag ata ctc 2294
Ile Ile Leu Gly Leu Pro Val Ser Ala Arg Arg Gly Lys Glu Ile Leu
370 375 380

ctg ggc ccg gcc gat agt ctt gaa ggg cgg ggg tgg cga ctc ctc gcg 2342
Leu Gly Pro Ala Asp Ser Leu Glu Gly Arg Gly Trp Arg Leu Leu Ala
385 390 395

ccc atc acg gcc tac tcc caa cag acg cgg ggc cta ctt ggt tgc atc 2390
Pro Ile Thr Ala Tyr Ser Gln Gln Thr Arg Gly Leu Leu Gly Cys Ile
400 405 410

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atc act agc ctt aca ggc cgg gac aag aac cag gtc gag gga gag gtt    2438
Ile Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu Gly Glu Val
415                 420                 425                 430

cag gtg gtt tcc acc gca aca caa tcc ttc ctg gcg acc tgc gtc aac    2486
Gln Val Val Ser Thr Ala Thr Gln Ser Phe Leu Ala Thr Cys Val Asn
                435                 440                 445

ggc gtg tgt tgg acc gtt tac cat ggt gct ggc tca aag acc tta gcc    2534
Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly Ser Lys Thr Leu Ala
            450                 455                 460

ggc cca aag ggg cca atc acc cag atg tac act aat gtg gac cag gac    2582
Gly Pro Lys Gly Pro Ile Thr Gln Met Tyr Thr Asn Val Asp Gln Asp
        465                 470                 475

ctc gtc ggc tgg cag gcg ccc ccc ggg gcg cgt tcc ttg aca cca tgc    2630
Leu Val Gly Trp Gln Ala Pro Pro Gly Ala Arg Ser Leu Thr Pro Cys
    480                 485                 490

acc tgt ggc agc tca gac ctt tac ttg gtc acg aga cat gct gac gtc    2678
Thr Cys Gly Ser Ser Asp Leu Tyr Leu Val Thr Arg His Ala Asp Val
495                 500                 505                 510

att ccg gtg cgc cgg cgg ggc gac agt agg ggg agc ctg ctc tcc ccc    2726
Ile Pro Val Arg Arg Arg Gly Asp Ser Arg Gly Ser Leu Leu Ser Pro
                515                 520                 525

agg cct gtc tcc tac ttg aag ggc tct gcg ggt ggt cca ctg ctc tgc    2774
Arg Pro Val Ser Tyr Leu Lys Gly Ser Ala Gly Gly Pro Leu Leu Cys
            530                 535                 540

cct tcg ggg cac gct gtg ggc atc ttc cgg gct gcc gta tgc acc cgg    2822
Pro Ser Gly His Ala Val Gly Ile Phe Arg Ala Ala Val Cys Thr Arg
        545                 550                 555

ggg gtt gcg aag gcg gtg gac ttt gtg ccc gta gag tcc atg gaa act    2870
Gly Val Ala Lys Ala Val Asp Phe Val Pro Val Glu Ser Met Glu Thr
    560                 565                 570

act atg cgg tct ccg gtc ttc acg gac aac tca tcc ccc ccg gcc gta    2918
Thr Met Arg Ser Pro Val Phe Thr Asp Asn Ser Ser Pro Pro Ala Val
575                 580                 585                 590

ccg cag tca ttt caa gtg gcc cac cta cac gct ccc act ggc agc ggc    2966
Pro Gln Ser Phe Gln Val Ala His Leu His Ala Pro Thr Gly Ser Gly
                595                 600                 605

aag agt act aaa gtg ccg gct gca tat gca gcc caa ggg tac aag gtg    3014
Lys Ser Thr Lys Val Pro Ala Ala Tyr Ala Ala Gln Gly Tyr Lys Val
            610                 615                 620

ctc gtc ctc aat ccg tcc gtt gcc gct acc tta ggg ttt ggg gcg tat    3062
Leu Val Leu Asn Pro Ser Val Ala Ala Thr Leu Gly Phe Gly Ala Tyr
        625                 630                 635

atg tct aag gca cac ggt att gac ccc aac atc aga act ggg gta agg    3110
Met Ser Lys Ala His Gly Ile Asp Pro Asn Ile Arg Thr Gly Val Arg
    640                 645                 650
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acc att acc aca ggc gcc ccc gtc aca tac tct acc tat ggc aag ttt 3158
Thr Ile Thr Thr Gly Ala Pro Val Thr Tyr Ser Thr Tyr Gly Lys Phe
655 660 665 670

ctt gcc gat ggt ggt tgc tct ggg ggc gct tat gac atc ata ata tgt 3206
Leu Ala Asp Gly Gly Cys Ser Gly Gly Ala Tyr Asp Ile Ile Ile Cys
675 680 685

gat gag tgc cat tca act gac tcg act aca atc ttg ggc atc ggc aca 3254
Asp Glu Cys His Ser Thr Asp Ser Thr Thr Ile Leu Gly Ile Gly Thr
690 695 700

gtc ctg gac caa gcg gag acg gct gga gcg cgg ctt gtc gtg ctc gcc 3302
Val Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Val Val Leu Ala
705 710 715

acc gct acg cct ccg gga tcg gtc acc gtg cca cac cca aac atc gag 3350
Thr Ala Thr Pro Pro Gly Ser Val Thr Val Pro His Pro Asn Ile Glu
720 725 730

gag gtg gcc ctg tct aat act gga gag atc ccc ttc tat ggc aaa gcc 3398
Glu Val Ala Leu Ser Asn Thr Gly Glu Ile Pro Phe Tyr Gly Lys Ala
735 740 745 750

atc ccc att gaa gcc atc agg ggg gga agg cat ctc att ttc tgt cat 3446
Ile Pro Ile Glu Ala Ile Arg Gly Gly Arg His Leu Ile Phe Cys His
755 760 765

tcc aag aag aag tgc gac gag ctc gcc gca aag ctg tca ggc ctc gga 3494
Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Ser Gly Leu Gly
770 775 780

atc aac gct gtg gcg tat tac cgg ggg ctc gat gtg tcc gtc ata cca 3542
Ile Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val Ile Pro
785 790 795

act atc gga gac gtc gtt gtc gtg gca aca gac gct ctg atg acg ggc 3590
Thr Ile Gly Asp Val Val Val Val Ala Thr Asp Ala Leu Met Thr Gly
800 805 810

tat acg ggc gac ttt gac tca gtg atc gac tgt aac aca tgt gtc acc 3638
Tyr Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Thr Cys Val Thr
815 820 825 830

cag aca gtc gac ttc agc ttg gat ccc acc ttc acc att gag acg acg 3686
Gln Thr Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile Glu Thr Thr
835 840 845

acc gtg cct caa gac gca gtg tcg cgc tcg cag cgg cgg ggt agg act 3734
Thr Val Pro Gln Asp Ala Val Ser Arg Ser Gln Arg Arg Gly Arg Thr
850 855 860

ggc agg ggt agg aga ggc atc tac agg ttt gtg act ccg gga gaa cgg 3782
Gly Arg Gly Arg Arg Gly Ile Tyr Arg Phe Val Thr Pro Gly Glu Arg
865 870 875

ccc tcg ggc atg ttc gat tcc tcg gtc ctg tgt gag tgc tat gac gcg 3830
Pro Ser Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys Tyr Asp Ala
880 885 890

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ggc tgt gct tgg tac gag ctc acc ccc gcc gag acc tcg gtt agg ttg     3878
Gly Cys Ala Trp Tyr Glu Leu Thr Pro Ala Glu Thr Ser Val Arg Leu
895                 900                 905                 910

cgg gcc tac ctg aac aca cca ggg ttg ccc gtt tgc cag gac cac ctg     3926
Arg Ala Tyr Leu Asn Thr Pro Gly Leu Pro Val Cys Gln Asp His Leu
                915                 920                 925

gag ttc tgg gag agt gtc ttc aca ggc ctc acc cat ata gat gca cac     3974
Glu Phe Trp Glu Ser Val Phe Thr Gly Leu Thr His Ile Asp Ala His
            930                 935                 940

ttc ttg tcc cag acc aag cag gca gga gac aac ttc ccc tac ctg gta     4022
Phe Leu Ser Gln Thr Lys Gln Ala Gly Asp Asn Phe Pro Tyr Leu Val
        945                 950                 955

gca tac caa gcc acg gtg tgc gcc agg gct cag gcc cca cct cca tca     4070
Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro Pro Pro Ser
    960                 965                 970

tgg gat caa atg tgg aag tgt ctc ata cgg ctg aaa cct acg ctg cac     4118
Trp Asp Gln Met Trp Lys Cys Leu Ile Arg Leu Lys Pro Thr Leu His
975                 980                 985                 990

ggg cca aca ccc ttg ctg tac agg ctg gga gcc gtc caa aat gag gtc     4166
Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln Asn Glu Val
                995                1000                1005

acc ctc acc cac ccc ata acc aaa tac atc atg gca tgc atg tcg gct     4214
Thr Leu Thr His Pro Ile Thr Lys Tyr Ile Met Ala Cys Met Ser Ala
           1010                1015                1020

gac ctg gag gtc gtc act agc acc tgg gtg ctg gtg ggc gga gtc ctt     4262
Asp Leu Glu Val Val Thr Ser Thr Trp Val Leu Val Gly Gly Val Leu
       1025                1030                1035

gca gct ctg gcc gcg tat tgc ctg aca aca ggc agt gtg gtc att gtg     4310
Ala Ala Leu Ala Ala Tyr Cys Leu Thr Thr Gly Ser Val Val Ile Val
   1040                1045                1050

ggt agg att atc ttg tcc ggg agg ccg gcc att gtt ccc gac agg gag     4358
Gly Arg Ile Ile Leu Ser Gly Arg Pro Ala Ile Val Pro Asp Arg Glu
1055               1060                1065                1070

ctt ctc tac cag gag ttc gat gaa atg gaa gag tgc gcc tcg cac ctc     4406
Leu Leu Tyr Gln Glu Phe Asp Glu Met Glu Glu Cys Ala Ser His Leu
               1075                1080                1085

cct tac atc gag cag gga atg cag ctc gcc gag caa ttc aag cag aaa     4454
Pro Tyr Ile Glu Gln Gly Met Gln Leu Ala Glu Gln Phe Lys Gln Lys
           1090                1095                1100

gcg ctc ggg tta ctg caa aca gcc acc aaa caa gcg gag gct gct gct     4502
Ala Leu Gly Leu Leu Gln Thr Ala Thr Lys Gln Ala Glu Ala Ala Ala
       1105                1110                1115

ccc gtg gtg gag tcc aag tgg cga gcc ctt gag aca ttc tgg gcg aag     4550
Pro Val Val Glu Ser Lys Trp Arg Ala Leu Glu Thr Phe Trp Ala Lys
   1120                1125                1130
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cac atg tgg aat ttc atc agc ggg ata cag tac tta gca ggc tta tcc    4598
His Met Trp Asn Phe Ile Ser Gly Ile Gln Tyr Leu Ala Gly Leu Ser
1135                1140                1145                1150

act ctg cct ggg aac ccc gca ata gca tca ttg atg gca ttc aca gcc    4646
Thr Leu Pro Gly Asn Pro Ala Ile Ala Ser Leu Met Ala Phe Thr Ala
                1155                1160                1165

tct atc acc agc ccg ctc acc acc caa agt acc ctc ctg ttt aac atc    4694
Ser Ile Thr Ser Pro Leu Thr Thr Gln Ser Thr Leu Leu Phe Asn Ile
            1170                1175                1180

ttg ggg ggg tgg gtg gct gcc caa ctc gcc ccc ccc agc gcc gct tcg    4742
Leu Gly Gly Trp Val Ala Ala Gln Leu Ala Pro Pro Ser Ala Ala Ser
        1185                1190                1195

gct ttc gtg ggc gcc ggc atc gcc ggt gcg gct gtt ggc agc ata ggc    4790
Ala Phe Val Gly Ala Gly Ile Ala Gly Ala Ala Val Gly Ser Ile Gly
    1200                1205                1210

ctt ggg aag gtg ctt gtg gac att ctg gcg ggt tat gga gca gga gtg    4838
Leu Gly Lys Val Leu Val Asp Ile Leu Ala Gly Tyr Gly Ala Gly Val
1215                1220                1225                1230

gcc ggc gcg ctc gtg gcc ttt aag gtc atg agc ggc gag atg ccc tcc    4886
Ala Gly Ala Leu Val Ala Phe Lys Val Met Ser Gly Glu Met Pro Ser
                1235                1240                1245

acc gag gac ctg gtc aat cta ctt cct gcc atc ctc gag gaa gct agt    4934
Thr Glu Asp Leu Val Asn Leu Leu Pro Ala Ile Leu Glu Glu Ala Ser
            1250                1255                1260

gag gat gtc gtc tgc tgc tca atg tcc tac aca tgg aca ggc gcc ttg    4982
Glu Asp Val Val Cys Cys Ser Met Ser Tyr Thr Trp Thr Gly Ala Leu
        1265                1270                1275

gag ctg ctg ctg ctg ctg ctg ctg ggc ctg agg cta cag ctc tcc ctg    5030
Glu Leu Leu Leu Leu Leu Leu Leu Gly Leu Arg Leu Gln Leu Ser Leu
    1280                1285                1290

ggc atc atc cca gtt gag gag gag aac ccg gac ttc tgg aac cgc gag    5078
Gly Ile Ile Pro Val Glu Glu Glu Asn Pro Asp Phe Trp Asn Arg Glu
1295                1300                1305                1310

gca gcc gag gcc ctg ggt gcc gcc aag aag ctg cag cct gca cag aca    5126
Ala Ala Glu Ala Leu Gly Ala Ala Lys Lys Leu Gln Pro Ala Gln Thr
                1315                1320                1325

gcc gcc aag aac ctc atc atc ttc ctg ggc gat ggg atg ggg gtg tct    5174
Ala Ala Lys Asn Leu Ile Ile Phe Leu Gly Asp Gly Met Gly Val Ser
            1330                1335                1340

acg gtg aca gct gcc agg atc cta aaa ggg cag aag aag gac aaa ctg    5222
Thr Val Thr Ala Ala Arg Ile Leu Lys Gly Gln Lys Lys Asp Lys Leu
        1345                1350                1355

ggg cct gag ata ccc ctg gcc atg gac cgc ttc cca tat gtg gct ctg    5270
Gly Pro Glu Ile Pro Leu Ala Met Asp Arg Phe Pro Tyr Val Ala Leu
    1360                1365                1370
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tcc aag aca tac aat gta gac aaa cat gtg cca gac agt gga gcc aca 5318
Ser Lys Thr Tyr Asn Val Asp Lys His Val Pro Asp Ser Gly Ala Thr
1375 1380 1385 1390

gcc acg gcc tac ctg tgc ggg gtc aag ggc aac ttc cag acc att ggc 5366
Ala Thr Ala Tyr Leu Cys Gly Val Lys Gly Asn Phe Gln Thr Ile Gly
1395 1400 1405

ttg agt gca gcc gcc cgc ttt aac cag tgc aac acg aca cgc ggc aac 5414
Leu Ser Ala Ala Ala Arg Phe Asn Gln Cys Asn Thr Thr Arg Gly Asn
1410 1415 1420

gag gtc atc tcc gtg atg aat cgg gcc aag aaa gca ggg aag tca gtg 5462
Glu Val Ile Ser Val Met Asn Arg Ala Lys Lys Ala Gly Lys Ser Val
1425 1430 1435

gga gtg gta acc acc aca cga gtg cag cac gcc tcg cca gcc ggc acc 5510
Gly Val Val Thr Thr Thr Arg Val Gln His Ala Ser Pro Ala Gly Thr
1440 1445 1450

tac gcc cac acg gtg aac cgc aac tgg tac tcg gac gcc gac gtg cct 5558
Tyr Ala His Thr Val Asn Arg Asn Trp Tyr Ser Asp Ala Asp Val Pro
1455 1460 1465 1470

gcc tcg gcc cgc cag gag ggg tgc cag gac atc gct acg cag ctc atc 5606
Ala Ser Ala Arg Gln Glu Gly Cys Gln Asp Ile Ala Thr Gln Leu Ile
1475 1480 1485

tcc aac atg gac att gac gtg atc cta ggt gga ggc cga aag tac atg 5654
Ser Asn Met Asp Ile Asp Val Ile Leu Gly Gly Gly Arg Lys Tyr Met
1490 1495 1500

ttt ccc atg gga acc cca gac cct gag tac cca gat gac tac agc caa 5702
Phe Pro Met Gly Thr Pro Asp Pro Glu Tyr Pro Asp Asp Tyr Ser Gln
1505 1510 1515

ggt ggg acc agg ctg gac ggg aag aat ctg gtg cag gaa tgg ctg gcg 5750
Gly Gly Thr Arg Leu Asp Gly Lys Asn Leu Val Gln Glu Trp Leu Ala
1520 1525 1530

aag cgc cag ggt gcc cgg tat gtg tgg aac cgc act gag ctg atg cag 5798
Lys Arg Gln Gly Ala Arg Tyr Val Trp Asn Arg Thr Glu Leu Met Gln
1535 1540 1545 1550

gct tcc ctg gac ccg tct gtg acc cat ctc atg ggt ctc ttt gag cct 5846
Ala Ser Leu Asp Pro Ser Val Thr His Leu Met Gly Leu Phe Glu Pro
1555 1560 1565

gga gac atg aaa tac gag atc cac cga gac tcc aca ctg gac ccc tcc 5894
Gly Asp Met Lys Tyr Glu Ile His Arg Asp Ser Thr Leu Asp Pro Ser
1570 1575 1580

ctg atg gag atg aca gag gct gcc ctg cgc ctg ctg agc agg aac ccc 5942
Leu Met Glu Met Thr Glu Ala Ala Leu Arg Leu Leu Ser Arg Asn Pro
1585 1590 1595

cgc ggc ttc ttc ctc ttc gtg gag ggt ggt cgc atc gac cat ggt cat 5990
Arg Gly Phe Phe Leu Phe Val Glu Gly Gly Arg Ile Asp His Gly His
1600 1605 1610

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cat gaa agc agg gct tac cgg gca ctg act gag acg atc atg ttc gac    6038
His Glu Ser Arg Ala Tyr Arg Ala Leu Thr Glu Thr Ile Met Phe Asp
1615                1620                1625                1630

gac gcc att gag agg gcg ggc cag ctc acc agc gag gag gac acg ctg    6086
Asp Ala Ile Glu Arg Ala Gly Gln Leu Thr Ser Glu Glu Asp Thr Leu
                1635                1640                1645

agc ctc gtc act gcc gac cac tcc cac gtc ttc tcc ttc gga ggc tac    6134
Ser Leu Val Thr Ala Asp His Ser His Val Phe Ser Phe Gly Gly Tyr
            1650                1655                1660

ccc ctg cga ggg agc tgc atc ttc ggg ctg gcc cct ggc aag gcc cgg    6182
Pro Leu Arg Gly Ser Cys Ile Phe Gly Leu Ala Pro Gly Lys Ala Arg
        1665                1670                1675

gac agg aag gcc tac acg gtc ctc cta tac gga aac ggt cca ggc tat    6230
Asp Arg Lys Ala Tyr Thr Val Leu Leu Tyr Gly Asn Gly Pro Gly Tyr
    1680                1685                1690

gtg ctc aag gac ggc gcc cgg ccg gat gtt acc gag agc gag agc ggg    6278
Val Leu Lys Asp Gly Ala Arg Pro Asp Val Thr Glu Ser Glu Ser Gly
1695                1700                1705                1710

agc ccc gag tat cgg cag cag tca gca gtg ccc ctg gac gaa gag acc    6326
Ser Pro Glu Tyr Arg Gln Gln Ser Ala Val Pro Leu Asp Glu Glu Thr
                1715                1720                1725

cac gca ggc gag gac gtg gcg gtg ttc gcg cgc ggc ccg cag gcg cac    6374
His Ala Gly Glu Asp Val Ala Val Phe Ala Arg Gly Pro Gln Ala His
            1730                1735                1740

ctg gtt cac ggc gtg cag gag cag acc ttc ata gcg cac gtc atg gcc    6422
Leu Val His Gly Val Gln Glu Gln Thr Phe Ile Ala His Val Met Ala
        1745                1750                1755

ttc gcc gcc tgc ctg gag ccc tac acc gcc tgc gac ctg gcg ccc ccc    6470
Phe Ala Ala Cys Leu Glu Pro Tyr Thr Ala Cys Asp Leu Ala Pro Pro
    1760                1765                1770

gcc ggc acc acc gac gcc gcg cac ccg ggt taacccgtgg tccccgcgtt      6520
Ala Gly Thr Thr Asp Ala Ala His Pro Gly
1775                1780

gcttcctctg ctggccggga catcaggtgg cccccgctga attggaatcg atattgttac 6580

aacaccccaa catcttcgac gcgggcgtgg caggtcttcc cgacgatgac gccggtgaac 6640

ttcccgccgc cgttgttgtt ttggagcacg gaaagacgat gacggaaaaa gagatcgtgg 6700

attacgtcgc cagtcaagta acaaccgcga aaaagttgcg cggaggagtt gtgtttgtgg 6760

acgaagtacc gaaaggtctt accggaaaac tcgacgcaag aaaaatcaga gagatcctca 6820

taaaggccaa gaagggcgga aagtccaaat tgtaaaatgt aactgtattc agcgatgacg 6880

aaattcttag ctattgtaat actgcgatga gtggcagggc ggggcgtaat ttttttaagg 6940

cagttattgg tgcccttaaa cgcctggtgc tacgcctgaa taagtgataa taagcggatg 7000

aatggcagaa attcgccgga tctttgtgaa ggaaccttac ttctgtggtg tgacataatt 7060
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ggacaaacta cctacagaga tttaaagctc taaggtaaat ataaaatttt taagtgtata 7120
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ctaatttatt gcacggtaag gaagtagaat cataaagaac agtgacggat cgatccccca 8380
agcttggaca caagacaggc ttgcgagata tgtttgagaa taccacttta tcccgcgtca 8440
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gatctctata atctcgcgca acctattttc ccctcgaaca ctttttaagc cgtagataaa 8560
caggctggga cacttcac atg agc gaa aaa tac atc gtc acc tgg gac atg    8611
                   Met Ser Glu Lys Tyr Ile Val Thr Trp Asp Met
                   1785                1790                1795

ttg cag atc cat gca cgt aaa ctc gca agc cga ctg atg cct tct gaa    8659
Leu Gln Ile His Ala Arg Lys Leu Ala Ser Arg Leu Met Pro Ser Glu
               1800                1805                1810
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caa tgg aaa ggc att att gcc gta agc cgt ggc ggt ctg gta ccg ggt    8707
Gln Trp Lys Gly Ile Ile Ala Val Ser Arg Gly Gly Leu Val Pro Gly
            1815                1820                1825

gcg tta ctg gcg cgt gaa ctg ggt att cgt cat gtc gat acc gtt tgt    8755
Ala Leu Leu Ala Arg Glu Leu Gly Ile Arg His Val Asp Thr Val Cys
        1830                1835                1840

att tcc agc tac gat cac gac aac cag cgc gag ctt aaa gtg ctg aaa    8803
Ile Ser Ser Tyr Asp His Asp Asn Gln Arg Glu Leu Lys Val Leu Lys
    1845                1850                1855

cgc gca gaa ggc gat ggc gaa ggc ttc atc gtt att gat gac ctg gtg    8851
Arg Ala Glu Gly Asp Gly Glu Gly Phe Ile Val Ile Asp Asp Leu Val
1860                1865                1870                1875

gat acc ggt ggt act gcg gtt gcg att cgt gaa atg tat cca aaa gcg    8899
Asp Thr Gly Gly Thr Ala Val Ala Ile Arg Glu Met Tyr Pro Lys Ala
                1880                1885                1890

cac ttt gtc acc atc ttc gca aaa ccg gct ggt cgt ccg ctg gtt gat    8947
His Phe Val Thr Ile Phe Ala Lys Pro Ala Gly Arg Pro Leu Val Asp
            1895                1900                1905

gac tat gtt gtt gat atc ccg caa gat acc tgg att gaa cag ccg tgg    8995
Asp Tyr Val Val Asp Ile Pro Gln Asp Thr Trp Ile Glu Gln Pro Trp
        1910                1915                1920

gat atg ggc gtc gta ttc gtc ccg cca atc tcc ggt cgc taatcttttc     9044
Asp Met Gly Val Val Phe Val Pro Pro Ile Ser Gly Arg
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aacgcctggc actgccgggc gttgttcttt ttaacttcag gcgggttaca atagtttcca 9104

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gatcaa ttc tgt gag cgt atg gca aac gaa gga aaa ata gtt ata gta     10232
       Phe Cys Glu Arg Met Ala Asn Glu Gly Lys Ile Val Ile Val
                1940                1945                1950

gcc gca ctc gat ggg aca ttt caa cgt aaa ccg ttt aat aat att ttg    10280
Ala Ala Leu Asp Gly Thr Phe Gln Arg Lys Pro Phe Asn Asn Ile Leu
            1955                1960                1965

aat ctt att cca tta tct gaa atg gtg gta aaa cta act gct gtg tgt    10328
Asn Leu Ile Pro Leu Ser Glu Met Val Val Lys Leu Thr Ala Val Cys
        1970                1975                1980

atg aaa tgc ttt aag gag gct tcc ttt tct aaa cga ttg ggt gag gaa    10376
Met Lys Cys Phe Lys Glu Ala Ser Phe Ser Lys Arg Leu Gly Glu Glu
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acc gag ata gaa ata ata gga ggt aat gat atg tat caa tcg gtg tgt    10424
Thr Glu Ile Glu Ile Ile Gly Gly Asn Asp Met Tyr Gln Ser Val Cys
2000                2005                2010

aga aag tgt tac atc gac tca taatattata tttttatct aaaaaactaa       10475
Arg Lys Cys Tyr Ile Asp Ser
2015                2020

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Ile Pro Phe Phe Ala Ala Phe Cys Leu Pro Val Phe Ala His Pro Glu
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Thr Leu Val Lys Val Lys Asp Ala Glu Asp Gln Leu Gly Ala Arg Val
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Arg Arg Ile His Tyr Ser Gln Asn Asp Leu Val Glu Tyr Ser Pro Val
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Thr Glu Lys His Leu Thr Asp Gly Met Thr Val Arg Glu Leu Cys Ser
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gct gcc ata acc atg agt gat aac act gcg gcc aac tta ctt ctg aca 12290
Ala Ala Ile Thr Met Ser Asp Asn Thr Ala Ala Asn Leu Leu Leu Thr
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Asp His Val Thr Arg Leu Asp Arg Trp Glu Pro Glu Leu Asn Glu Ala
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Gln Gln Leu Ile Asp Trp Met Glu Ala Asp Lys Val Ala Gly Pro Leu
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ctg cgc tcg gcc ctt ccg gct ggc tgg ttt att gct gat aaa tct gga    12578
Leu Arg Ser Ala Leu Pro Ala Gly Trp Phe Ile Ala Asp Lys Ser Gly
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Ala Gly Glu Arg Gly Ser Arg Gly Ile Ile Ala Ala Leu Gly Pro Asp
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Gly Lys Pro Ser Arg Ile Val Val Ile Tyr Thr Thr Gly Ser Gln Ala
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Ile Lys His Trp
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Tyr Lys Cys Val Thr Ile Lys Tyr Ser Asn Asp Asn Arg Tyr Gly Thr
        35   40   45

Gly Leu Trp Thr His Asp Lys Asn Asn Phe Glu Ala Leu Glu Ala Thr
    50   55   60

Lys Leu Cys Asp Val Leu Glu Ser Ile Thr Asp Phe Ser Val Ile Gly
65   70   75   80

Ile Asp Glu Gly Gln Phe Phe Pro Asp Ile Val Glu Met Gly Ile Pro
                85   90   95

Gln Phe Met Ala Arg Val Cys Ala Cys Leu Trp Met Met Leu Leu Ile
            100   105   110

Ala Gln Ala Glu Ala Ala Leu Glu Asn Leu Val Val Leu Asn Ala Ala
        115   120   125

Ser Val Ala Gly Ala His Gly Ile Leu Ser Phe Leu Val Phe Phe Cys
    130   135   140

Ala Ala Trp Tyr Ile Lys Gly Arg Leu Val Pro Gly Ala Ala Tyr Ala
145   150   155   160

Leu Tyr Gly Val Trp Pro Leu Leu Leu Leu Leu Leu Ala Leu Pro Pro
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Arg Ala Tyr Ala Met Asp Arg Glu Met Ala Ala Ser Cys Gly Gly Ala
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Val Phe Val Gly Leu Val Leu Leu Thr Leu Ser Pro Tyr Tyr Lys Val
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Phe Leu Ala Arg Leu Ile Trp Trp Leu Gln Tyr Phe Thr Thr Arg Ala
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Glu Ala His Leu His Val Trp Ile Pro Pro Leu Asn Ala Arg Gly Gly
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Phe Asp Ile Thr Lys Leu Leu Ile Ala Ile Leu Gly Pro Leu Met Val
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Val Gln Met Ala Phe Met Lys Leu Gly Ala Leu Thr Gly Thr Tyr Ile
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Tyr Asn His Leu Thr Pro Leu Arg Asp Trp Ala His Ala Gly Leu Arg
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Lys Ile Ile Thr Trp Gly Ala Asp Thr Ala Ala Ala Gly Asp Ile Ile
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Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu Gly Glu Val Gln Val
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Thr Lys Val Pro Ala Ala Tyr Ala Ala Gln Gly Tyr Lys Val Leu Val
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Lys Ala His Gly Ile Asp Pro Asn Ile Arg Thr Gly Val Arg Thr Ile
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Thr Thr Gly Ala Pro Val Thr Tyr Ser Thr Tyr Gly Lys Phe Leu Ala
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Asp Gly Gly Cys Ser Gly Gly Ala Tyr Asp Ile Ile Ile Cys Asp Glu
        675                 680                 685

Cys His Ser Thr Asp Ser Thr Thr Ile Leu Gly Ile Gly Thr Val Leu
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Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Val Val Leu Ala Thr Ala
705                 710                 715                 720

Thr Pro Pro Gly Ser Val Thr Val Pro His Pro Asn Ile Glu Glu Val
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Ala Leu Ser Asn Thr Gly Glu Ile Pro Phe Tyr Gly Lys Ala Ile Pro
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Ile Glu Ala Ile Arg Gly Gly Arg His Leu Ile Phe Cys His Ser Lys
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Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Ser Gly Leu Gly Ile Asn
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Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile Glu Thr Thr Thr Val
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Trp Glu Ser Val Phe Thr Gly Leu Thr His Ile Asp Ala His Phe Leu
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Ser Gln Thr Lys Gln Ala Gly Asp Asn Phe Pro Tyr Leu Val Ala Tyr
945 950 955 960

Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro Pro Pro Ser Trp Asp
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Gln Met Trp Lys Cys Leu Ile Arg Leu Lys Pro Thr Leu His Gly Pro
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Thr His Pro Ile Thr Lys Tyr Ile Met Ala Cys Met Ser Ala Asp Leu
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Ile Glu Gln Gly Met Gln Leu Ala Glu Gln Phe Lys Gln Lys Ala Leu
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Gly Leu Leu Gln Thr Ala Thr Lys Gln Ala Glu Ala Ala Ala Pro Val
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Val Glu Ser Lys Trp Arg Ala Leu Glu Thr Phe Trp Ala Lys His Met
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1810 1815 1820

Val Pro Gly Ala Leu Leu Ala Arg Glu Leu Gly Ile Arg His Val Asp
825 1830 1835 1840

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1845 1850 1855

Val Leu Lys Arg Ala Glu Gly Asp Gly Glu Gly Phe Ile Val Ile Asp
1860 1865 1870

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1875 1880 1885

Pro Lys Ala His Phe Val Thr Ile Phe Ala Lys Pro Ala Gly Arg Pro
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Gln Pro Trp Asp Met Gly Val Val Phe Val Pro Pro Ile Ser Gly Arg
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1940 1945 1950

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1970 1975 1980

Cys Phe Lys Glu Ala Ser Phe Ser Lys Arg Leu Gly Glu Glu Thr Glu
985 1990 1995 2000

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Arg Ile His Tyr Ser Gln Asn Asp Leu Val Glu Tyr Ser Pro Val Thr
2115 2120 2125

Glu Lys His Leu Thr Asp Gly Met Thr Val Arg Glu Leu Cys Ser Ala
2130 2135 2140

Ala Ile Thr Met Ser Asp Asn Thr Ala Ala Asn Leu Leu Leu Thr Thr
145 2150 2155 216

Ile Gly Gly Pro Lys Glu Leu Thr Ala Phe Leu His Asn Met Gly Asp
2165 2170 2175

His Val Thr Arg Leu Asp Arg Trp Glu Pro Glu Leu Asn Glu Ala Ile
2180 2185 2190

Pro Asn Asp Glu Arg Asp Thr Thr Met Pro Val Ala Met Ala Thr Thr
2195 2200 2205

Leu Arg Lys Leu Leu Thr Gly Glu Leu Leu Thr Leu Ala Ser Arg Gln
2210 2215 2220

Gln Leu Ile Asp Trp Met Glu Ala Asp Lys Val Ala Gly Pro Leu Leu
225 2230 2235 224

Arg Ser Ala Leu Pro Ala Gly Trp Phe Ile Ala Asp Lys Ser Gly Ala
2245 2250 2255

Gly Glu Arg Gly Ser Arg Gly Ile Ile Ala Ala Leu Gly Pro Asp Gly
2260 2265 2270

Lys Pro Ser Arg Ile Val Val Ile Tyr Thr Thr Gly Ser Gln Ala Thr
2275 2280 2285

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2290 2295 2300

Lys His Trp
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<210> 17
<211> 92
<212> PRT
<213> Artificial Sequence

<400> 17
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1 5 10 15

Lys Ser Thr Glu Leu Ile Arg Arg Val Arg Arg Tyr Gln Ile Ala Gln
20 25 30

Tyr Lys Cys Val Thr Ile Lys Tyr Ser Asn Asp Asn Arg Tyr Gly Thr
35 40 45

Gly Leu Trp Thr His Asp Lys Asn Asn Phe Glu Ala Leu Glu Ala Thr
50 55 60

Lys Leu Cys Asp Val Leu Glu Ser Ile Thr Asp Phe Ser Val Ile Gly
65 70 75 80

Ile Asp Glu Gly Gln Phe Phe Pro Asp Ile Val Glu
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<210> 18
<211> 1692
<212> PRT
<213> Artificial Sequence

<400> 18

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 1               5                  10                  15

Met Leu Leu Ile Ala Gln Ala Glu Ala Ala Leu Glu Asn Leu Val Val
            20                  25                  30

Leu Asn Ala Ala Ser Val Ala Gly Ala His Gly Ile Leu Ser Phe Leu
        35                  40                  45

Val Phe Phe Cys Ala Ala Trp Tyr Ile Lys Gly Arg Leu Val Pro Gly
    50                  55                  60

Ala Ala Tyr Ala Leu Tyr Gly Val Trp Pro Leu Leu Leu Leu Leu Leu
65                  70                  75                  80

Ala Leu Pro Pro Arg Ala Tyr Ala Met Asp Arg Glu Met Ala Ala Ser
                85                  90                  95

Cys Gly Gly Ala Val Phe Val Gly Leu Val Leu Leu Thr Leu Ser Pro
            100                 105                 110

Tyr Tyr Lys Val Phe Leu Ala Arg Leu Ile Trp Trp Leu Gln Tyr Phe
        115                 120                 125

Thr Thr Arg Ala Glu Ala His Leu His Val Trp Ile Pro Pro Leu Asn
    130                 135                 140

Ala Arg Gly Gly Arg Asp Ala Ile Ile Leu Leu Met Cys Ala Val His
145                 150                 155                 160

Pro Glu Leu Ile Phe Asp Ile Thr Lys Leu Leu Ile Ala Ile Leu Gly
                165                 170                 175

Pro Leu Met Val Leu Gln Ala Gly Ile Thr Arg Val Pro Tyr Phe Val
            180                 185                 190

Arg Ala Gln Gly Leu Ile His Ala Cys Met Leu Val Arg Lys Val Ala
        195                 200                 205

Gly Gly His Tyr Val Gln Met Ala Phe Met Lys Leu Gly Ala Leu Thr
    210                 215                 220

Gly Thr Tyr Ile Tyr Asn His Leu Thr Pro Leu Arg Asp Trp Ala His
225                 230                 235                 240

Ala Gly Leu Arg Asp Leu Ala Val Ala Val Glu Pro Val Val Phe Ser
                245                 250                 255

Asp Met Glu Thr Lys Ile Ile Thr Trp Gly Ala Asp Thr Ala Ala Ala
            260                 265                 270

Gly Asp Ile Ile Leu Gly Leu Pro Val Ser Ala Arg Arg Gly Lys Glu
        275                 280                 285

Ile Leu Leu Gly Pro Ala Asp Ser Leu Glu Gly Arg Gly Trp Arg Leu
    290                 295                 300

Leu Ala Pro Ile Thr Ala Tyr Ser Gln Gln Thr Arg Gly Leu Leu Gly
305                 310                 315                 320
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Cys Ile Ile Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu Gly
325 330 335

Glu Val Gln Val Val Ser Thr Ala Thr Gln Ser Phe Leu Ala Thr Cys
340 345 350

Val Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly Ser Lys Thr
355 360 365

Leu Ala Gly Pro Lys Gly Pro Ile Thr Gln Met Tyr Thr Asn Val Asp
370 375 380

Gln Asp Leu Val Gly Trp Gln Ala Pro Pro Gly Ala Arg Ser Leu Thr
385 390 395 400

Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr Leu Val Thr Arg His Ala
405 410 415

Asp Val Ile Pro Val Arg Arg Arg Gly Asp Ser Arg Gly Ser Leu Leu
420 425 430

Ser Pro Arg Pro Val Ser Tyr Leu Lys Gly Ser Ala Gly Gly Pro Leu
435 440 445

Leu Cys Pro Ser Gly His Ala Val Gly Ile Phe Arg Ala Ala Val Cys
450 455 460

Thr Arg Gly Val Ala Lys Ala Val Asp Phe Val Pro Val Glu Ser Met
465 470 475 480

Glu Thr Thr Met Arg Ser Pro Val Phe Thr Asp Asn Ser Ser Pro Pro
485 490 495

Ala Val Pro Gln Ser Phe Gln Val Ala His Leu His Ala Pro Thr Gly
500 505 510

Ser Gly Lys Ser Thr Lys Val Pro Ala Ala Tyr Ala Ala Gln Gly Tyr
515 520 525

Lys Val Leu Val Leu Asn Pro Ser Val Ala Ala Thr Leu Gly Phe Gly
530 535 540

Ala Tyr Met Ser Lys Ala His Gly Ile Asp Pro Asn Ile Arg Thr Gly
545 550 555 560

Val Arg Thr Ile Thr Thr Gly Ala Pro Val Thr Tyr Ser Thr Tyr Gly
565 570 575

Lys Phe Leu Ala Asp Gly Gly Cys Ser Gly Gly Ala Tyr Asp Ile Ile
580 585 590

Ile Cys Asp Glu Cys His Ser Thr Asp Ser Thr Thr Ile Leu Gly Ile
595 600 605

Gly Thr Val Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Val Val
610 615 620

Leu Ala Thr Ala Thr Pro Pro Gly Ser Val Thr Val Pro His Pro Asn
625 630 635 640

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Ile Glu Glu Val Ala Leu Ser Asn Thr Gly Glu Ile Pro Phe Tyr Gly
                645                 650                 655

Lys Ala Ile Pro Ile Glu Ala Ile Arg Gly Gly Arg His Leu Ile Phe
            660                 665                 670

Cys His Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Ser Gly
        675                 680                 685

Leu Gly Ile Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val
    690                 695                 700

Ile Pro Thr Ile Gly Asp Val Val Val Val Ala Thr Asp Ala Leu Met
705                 710                 715                 720

Thr Gly Tyr Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Thr Cys
                725                 730                 735

Val Thr Gln Thr Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile Glu
            740                 745                 750

Thr Thr Thr Val Pro Gln Asp Ala Val Ser Arg Ser Gln Arg Arg Gly
        755                 760                 765

Arg Thr Gly Arg Gly Arg Arg Gly Ile Tyr Arg Phe Val Thr Pro Gly
    770                 775                 780

Glu Arg Pro Ser Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys Tyr
785                 790                 795                 800

Asp Ala Gly Cys Ala Trp Tyr Glu Leu Thr Pro Ala Glu Thr Ser Val
                805                 810                 815

Arg Leu Arg Ala Tyr Leu Asn Thr Pro Gly Leu Pro Val Cys Gln Asp
            820                 825                 830

His Leu Glu Phe Trp Glu Ser Val Phe Thr Gly Leu Thr His Ile Asp
        835                 840                 845

Ala His Phe Leu Ser Gln Thr Lys Gln Ala Gly Asp Asn Phe Pro Tyr
    850                 855                 860

Leu Val Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro Pro
865                 870                 875                 880

Pro Ser Trp Asp Gln Met Trp Lys Cys Leu Ile Arg Leu Lys Pro Thr
                885                 890                 895

Leu His Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln Asn
            900                 905                 910

Glu Val Thr Leu Thr His Pro Ile Thr Lys Tyr Ile Met Ala Cys Met
        915                 920                 925

Ser Ala Asp Leu Glu Val Val Thr Ser Thr Trp Val Leu Val Gly Gly
    930                 935                 940

Val Leu Ala Ala Leu Ala Ala Tyr Cys Leu Thr Thr Gly Ser Val Val
945                 950                 955                 960
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Ile Val Gly Arg Ile Ile Leu Ser Gly Arg Pro Ala Ile Val Pro Asp
965 970 975

Arg Glu Leu Leu Tyr Gln Glu Phe Asp Glu Met Glu Glu Cys Ala Ser
980 985 990

His Leu Pro Tyr Ile Glu Gln Gly Met Gln Leu Ala Glu Gln Phe Lys
995 1000 1005

Gln Lys Ala Leu Gly Leu Leu Gln Thr Ala Thr Lys Gln Ala Glu Ala
1010 1015 1020

Ala Ala Pro Val Val Glu Ser Lys Trp Arg Ala Leu Glu Thr Phe Trp
025 1030 1035 1040

Ala Lys His Met Trp Asn Phe Ile Ser Gly Ile Gln Tyr Leu Ala Gly
1045 1050 1055

Leu Ser Thr Leu Pro Gly Asn Pro Ala Ile Ala Ser Leu Met Ala Phe
1060 1065 1070

Thr Ala Ser Ile Thr Ser Pro Leu Thr Thr Gln Ser Thr Leu Leu Phe
1075 1080 1085

Asn Ile Leu Gly Gly Trp Val Ala Ala Gln Leu Ala Pro Pro Ser Ala
1090 1095 1100

Ala Ser Ala Phe Val Gly Ala Gly Ile Ala Gly Ala Ala Val Gly Ser
105 1110 1115 1120

Ile Gly Leu Gly Lys Val Leu Val Asp Ile Leu Ala Gly Tyr Gly Ala
1125 1130 1135

Gly Val Ala Gly Ala Leu Val Ala Phe Lys Val Met Ser Gly Glu Met
1140 1145 1150

Pro Ser Thr Glu Asp Leu Val Asn Leu Leu Pro Ala Ile Leu Glu Glu
1155 1160 1165

Ala Ser Glu Asp Val Val Cys Cys Ser Met Ser Tyr Thr Trp Thr Gly
1170 1175 1180

Ala Leu Glu Leu Leu Leu Leu Leu Leu Leu Gly Leu Arg Leu Gln Leu
185 1190 1195 1200

Ser Leu Gly Ile Ile Pro Val Glu Glu Glu Asn Pro Asp Phe Trp Asn
1205 1210 1215

Arg Glu Ala Ala Glu Ala Leu Gly Ala Ala Lys Lys Leu Gln Pro Ala
1220 1225 1230

Gln Thr Ala Ala Lys Asn Leu Ile Ile Phe Leu Gly Asp Gly Met Gly
1235 1240 1245

Val Ser Thr Val Thr Ala Ala Arg Ile Leu Lys Gly Gln Lys Lys Asp
1250 1255 1260

Lys Leu Gly Pro Glu Ile Pro Leu Ala Met Asp Arg Phe Pro Tyr Val
265 1270 1275 1280

Ala Leu Ser Lys Thr Tyr Asn Val Asp Lys His Val Pro Asp Ser Gly
1285 1290 1295

Ala Thr Ala Thr Ala Tyr Leu Cys Gly Val Lys Gly Asn Phe Gln Thr
1300 1305 1310

Ile Gly Leu Ser Ala Ala Ala Arg Phe Asn Gln Cys Asn Thr Thr Arg
1315 1320 1325

Gly Asn Glu Val Ile Ser Val Met Asn Arg Ala Lys Lys Ala Gly Lys
1330 1335 1340

Ser Val Gly Val Val Thr Thr Thr Arg Val Gln His Ala Ser Pro Ala
345 1350 1355 1360

Gly Thr Tyr Ala His Thr Val Asn Arg Asn Trp Tyr Ser Asp Ala Asp
1365 1370 1375

Val Pro Ala Ser Ala Arg Gln Glu Gly Cys Gln Asp Ile Ala Thr Gln
1380 1385 1390

Leu Ile Ser Asn Met Asp Ile Asp Val Ile Leu Gly Gly Gly Arg Lys
1395 1400 1405

Tyr Met Phe Pro Met Gly Thr Pro Asp Pro Glu Tyr Pro Asp Asp Tyr
1410 1415 1420

Ser Gln Gly Gly Thr Arg Leu Asp Gly Lys Asn Leu Val Gln Glu Trp
425 1430 1435 1440

Leu Ala Lys Arg Gln Gly Ala Arg Tyr Val Trp Asn Arg Thr Glu Leu
1445 1450 1455

Met Gln Ala Ser Leu Asp Pro Ser Val Thr His Leu Met Gly Leu Phe
1460 1465 1470

Glu Pro Gly Asp Met Lys Tyr Glu Ile His Arg Asp Ser Thr Leu Asp
1475 1480 1485

Pro Ser Leu Met Glu Met Thr Glu Ala Ala Leu Arg Leu Leu Ser Arg
1490 1495 1500

Asn Pro Arg Gly Phe Phe Leu Phe Val Glu Gly Gly Arg Ile Asp His
505 1510 1515 1520

Gly His His Glu Ser Arg Ala Tyr Arg Ala Leu Thr Glu Thr Ile Met
1525 1530 1535

Phe Asp Asp Ala Ile Glu Arg Ala Gly Gln Leu Thr Ser Glu Glu Asp
1540 1545 1550

Thr Leu Ser Leu Val Thr Ala Asp His Ser His Val Phe Ser Phe Gly
1555 1560 1565

Gly Tyr Pro Leu Arg Gly Ser Cys Ile Phe Gly Leu Ala Pro Gly Lys
1570 1575 1580

Ala Arg Asp Arg Lys Ala Tyr Thr Val Leu Leu Tyr Gly Asn Gly Pro
585 1590 1595 1600

Gly Tyr Val Leu Lys Asp Gly Ala Arg Pro Asp Val Thr Glu Ser Glu
1605 1610 1615

Ser Gly Ser Pro Glu Tyr Arg Gln Gln Ser Ala Val Pro Leu Asp Glu
1620 1625 1630

Glu Thr His Ala Gly Glu Asp Val Ala Val Phe Ala Arg Gly Pro Gln
1635 1640 1645

Ala His Leu Val His Gly Val Gln Glu Gln Thr Phe Ile Ala His Val
1650 1655 1660

Met Ala Phe Ala Ala Cys Leu Glu Pro Tyr Thr Ala Cys Asp Leu Ala
665 1670 1675 1680

Pro Pro Ala Gly Thr Thr Asp Ala Ala His Pro Gly
1685 1690

<210> 19
<211> 152
<212> PRT
<213> Artificial Sequence

<400> 19
Met Ser Glu Lys Tyr Ile Val Thr Trp Asp Met Leu Gln Ile His Ala
1 5 10 15

Arg Lys Leu Ala Ser Arg Leu Met Pro Ser Glu Gln Trp Lys Gly Ile
20 25 30

Ile Ala Val Ser Arg Gly Gly Leu Val Pro Gly Ala Leu Leu Ala Arg
35 40 45

Glu Leu Gly Ile Arg His Val Asp Thr Val Cys Ile Ser Ser Tyr Asp
50 55 60

His Asp Asn Gln Arg Glu Leu Lys Val Leu Lys Arg Ala Glu Gly Asp
65 70 75 80

Gly Glu Gly Phe Ile Val Ile Asp Asp Leu Val Asp Thr Gly Gly Thr
85 90 95

Ala Val Ala Ile Arg Glu Met Tyr Pro Lys Ala His Phe Val Thr Ile
100 105 110

Phe Ala Lys Pro Ala Gly Arg Pro Leu Val Asp Asp Tyr Val Val Asp
115 120 125

Ile Pro Gln Asp Thr Trp Ile Glu Gln Pro Trp Asp Met Gly Val Val
130 135 140

Phe Val Pro Pro Ile Ser Gly Arg
145 150

<210> 20
<211> 85
<212> PRT
<213> Artificial Sequence

<400> 20
Phe Cys Glu Arg Met Ala Asn Glu Gly Lys Ile Val Ile Val Ala Ala
1 5 10 15

Leu Asp Gly Thr Phe Gln Arg Lys Pro Phe Asn Asn Ile Leu Asn Leu
20 25 30

Ile Pro Leu Ser Glu Met Val Val Lys Leu Thr Ala Val Cys Met Lys
35 40 45

Cys Phe Lys Glu Ala Ser Phe Ser Lys Arg Leu Gly Glu Glu Thr Glu
50 55 60

Ile Glu Ile Ile Gly Gly Asn Asp Met Tyr Gln Ser Val Cys Arg Lys
65 70 75 80

Cys Tyr Ile Asp Ser
85

<210> 21
<211> 286
<212> PRT
<213> Artificial Sequence

<400> 21
Met Ser Ile Gln His Phe Arg Val Ala Leu Ile Pro Phe Phe Ala Ala
1 5 10 15

Phe Cys Leu Pro Val Phe Ala His Pro Glu Thr Leu Val Lys Val Lys
20 25 30

Asp Ala Glu Asp Gln Leu Gly Ala Arg Val Gly Tyr Ile Glu Leu Asp
35 40 45

Leu Asn Ser Gly Lys Ile Leu Glu Ser Phe Arg Pro Glu Glu Arg Phe
50 55 60

Pro Met Met Ser Thr Phe Lys Val Leu Leu Cys Gly Ala Val Leu Ser
65 70 75 80

Arg Ile Asp Ala Gly Gln Glu Gln Leu Gly Arg Arg Ile His Tyr Ser
85 90 95

Gln Asn Asp Leu Val Glu Tyr Ser Pro Val Thr Glu Lys His Leu Thr
100 105 110

Asp Gly Met Thr Val Arg Glu Leu Cys Ser Ala Ala Ile Thr Met Ser
115 120 125

Asp Asn Thr Ala Ala Asn Leu Leu Leu Thr Thr Ile Gly Gly Pro Lys
130 135 140

Glu Leu Thr Ala Phe Leu His Asn Met Gly Asp His Val Thr Arg Leu
145 150 155 160

Asp Arg Trp Glu Pro Glu Leu Asn Glu Ala Ile Pro Asn Asp Glu Arg
165 170 175

Asp Thr Thr Met Pro Val Ala Met Ala Thr Thr Leu Arg Lys Leu Leu
180 185 190

Thr Gly Glu Leu Leu Thr Leu Ala Ser Arg Gln Gln Leu Ile Asp Trp
195 200 205

Met Glu Ala Asp Lys Val Ala Gly Pro Leu Leu Arg Ser Ala Leu Pro
210 215 220

Ala Gly Trp Phe Ile Ala Asp Lys Ser Gly Ala Gly Glu Arg Gly Ser
225 230 235 240

Arg Gly Ile Ile Ala Ala Leu Gly Pro Asp Gly Lys Pro Ser Arg Ile
245 250 255

Val Val Ile Tyr Thr Thr Gly Ser Gln Ala Thr Met Asp Glu Arg Asn
260 265 270

Arg Gln Ile Ala Glu Ile Gly Ala Ser Leu Ile Lys His Trp
275 280 285

<210> 22
<211> 220
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Sac 1/SEAP/Bam
H1 construct

<400> 22
gcgcgcgagc tcctgctgct gctgctgctg ggcctgaggc tacagctctc cctgggcatc 60

atcccagttg aggaggagaa cccggacttc tggaaccgcg aggcagccga ggccctgggt 120

gccgccaaga agctgcagcc tgcacagaca gccgccaaga acctcatcat cttcctgggc 180

gatgggatgg gggtgtctac ggtgacagct gccaggatcc 220

<210> 23
<211> 88
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: amino acid
fragment of the HCV polyprotein

<400> 23
Ala Arg Val Cys Ala Cys Leu Trp Met Met Leu Leu Ile Ala Gln Ala
1 5 10 15

Glu Ala Ala Leu Glu Asn Leu Val Val Leu Asn Ser Ala Ser Val Ala
20 25 30

Gly Ala His Gly Ile Leu Ser Phe Leu Val Phe Phe Cys Ala Ala Trp
35 40 45

Tyr Ile Lys Gly Arg Leu Val Pro Gly Ala Thr Tyr Ala Leu Tyr Gly
50 55 60

Val Trp Pro Leu Leu Leu Leu Leu Leu Ala Leu Pro Pro Arg Ala Tyr
65 70 75 80

Ala Met Asp Arg Glu Met Ala Ala
85

<210> 24
<211> 260
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: DNA fragment coding for an amino acid fragment of the HCV polyprotein

<400> 24
gcacgtgtct gtgcctgctt gtggatgatg ctgctgatag cccaggccga ggccgccttg 60

gagaacctgg tggtcctcaa tgcggcgtct gtggccggcg cacatggcat cctctccttc 120

cttgtgttct tctgtgccgc ctggtacatc aaaggcaggc tggtccctgg ggcggcatat 180

gctctttatg gcgtgtggcc gctgctcctg ctcttgctgg cattaccacc gcgagcttac 240

gccatggacc gggagatggc 260

<210> 25
<211> 177
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: amino acid fragment of the HCV polyprotein

<400> 25
Cys Ala Ser His Leu Pro Tyr Ile Glu Gln Gly Met Gln Leu Ala Glu
1 5 10 15

Gln Phe Lys Gln Lys Ala Leu Gly Leu Leu Gln Thr Ala Thr Lys Gln
20 25 30

Ala Glu Ala Ala Ala Pro Val Val Glu Ser Lys Trp Arg Ala Leu Glu
35 40 45

Thr Phe Trp Ala Lys His Met Trp Asn Phe Ile Ser Gly Ile Gln Tyr
50 55 60

Leu Ala Gly Leu Ser Thr Leu Pro Gly Asn Pro Ala Ile Ala Ser Leu
65 70 75 80

Met Ala Phe Thr Ala Ser Ile Thr Ser Pro Leu Thr Thr Gln Ser Thr
85 90 95

Leu Leu Phe Asn Ile Leu Gly Gly Trp Val Ala Ala Gln Leu Ala Pro
100 105 110

Pro Ser Ala Ala Ser Ala Phe Val Gly Ala Gly Ile Ala Gly Ala Ala
115 120 125

Val Gly Ser Ile Gly Leu Gly Lys Val Leu Val Asp Ile Leu Ala Gly
130 135 140

Tyr Gly Ala Gly Val Ala Gly Ala Leu Val Ala Phe Lys Val Met Ser
145 150 155 160

Gly Glu Met Pro Ser Thr Glu Asp Leu Val Asn Leu Leu Pro Ala Ile
165 170 175

Leu

<210> 26
<211> 528
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: DNA fragment coding for an amino acid fragment of the HCV polyprotein

<400> 26
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aaagcgctcg ggttactgca aacagccacc aaacaagcgg aggctgctgc tcccgtggtg 120

gagtccaagt ggcgagccct tgagacattc tgggcgaagc acatgtggaa tttcatcagc 180

gggatacagt acttagcagg cttatccact ctgcctggga accccgcaat agcatcattg 240

atggcattca cagcctctat caccagcccg ctcaccaccc aaagtaccct cctgtttaac 300

atcttggggg ggtgggtggc tgcccaactc gccccccccca gcgccgcttc ggctttcgtg 360

ggcgccggca tcgccggtgc ggctgttggc agcataggcc ttgggaaggt gcttgtggac 420

attctggcgg gttatggagc aggagtggcc ggcgcgctcg tggcctttaa ggtcatgagc 480

ggcgagatgc cctccaccga ggacctggtc aatctacttc ctgccatc 528

<210> 27
<211> 33
<212> DNA
<213> primer

<400> 27
gcgcgcgaat tcatggcacg tgtctgtgcc tgc 33

<210> 28
<211> 33
<212> DNA
<213> primer

<400> 28

cgcgcgctcg aggatggcag gaagtagatt gac 33

<210> 29
<211> 20
<212> PRT
<213> putative NS5A/5B cleavage site

<400> 29
Glu Glu Ala Ser Glu Asp Val Val Cys Cys Ser Met Ser Tyr Thr Trp
1 5 10 15

Thr Gly Ala Leu
20

<210> 30
<211> 33
<212> DNA
<213> primer

<400> 30
gcgcgcctcg aggaagctag tgaggatgtc gtc 33

<210> 31
<211> 36
<212> DNA
<213> primer

<400> 31
cgcgcggagc tccaaggcgc ctgtccatgt gtagga 36

<210> 32
<211> 69
<212> DNA
<213> primer

<400> 32
ctcgaggaag ctagtgagga tgtcgtctgc tgctcaatgt cctacacatg gacaggcgcc 60

ttggagctc 69

<210> 33
<211> 6
<212> PRT
<213> HCV/SEAP 6 amino acid fragment

<400> 33
Met Gly Ile Pro Gln Phe
1 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/17440

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :435/5, 6, 23, 320.1; 530/350; 536/23.2
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/5, 6, 23, 320.1; 530/350; 536/23.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST/ALL; Dialog

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HIROWATARI, Y. A Novel Method for Analysis of Viral Proteinase Activity Encoded by Hepatitis C Virus in Cultured Cells. Analytical Biochemistry. 1995, pages 113-120, see entire document.	1-41
Y	CHO, Y.-G. et al. In vivo assay for hepatitis C viral serine protease activity using a secreted protein. Journal of Virological Methods. 1998, Vol. 72, pages 109-115, see entire document.	1-41
Y	SONG, O-K. et al. Development of an in vivo Assay System Suitable for Screening Inhibitors of Hepatitis C Viral Protease. Molecular Cells. 1996, Vol. 6, No. 2, pages 183-189, see entire document.	1-41

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 NOVEMBER 1999	14 DEC 1999

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
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Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/17440

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,721,133 A (DASMAHAPATRA) 24 February 1998, see entire document.	1-41
A	US 5,739,002 A (DE FRANCESCO et al.) 14 April 1998.	1-41
A	INOUE, H. et al. Novel Assay System for Hepatitis C Virus Serine Protease Inhibitors. Antiviral Research. 1995, Vol. 26, No. 3. Abstract 122, page A289.	1-41

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/17440

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

G01N 33/576; C12Q 1/68; G03C 5/00; C12N 15/51; C07K 14/18; C07H 21/04